HERMODYNAMICS OF THE COMPLEXATION BETWEEN ASPARAGINE AND FIRST ROW TRANSITION METAL IONS : AN IN VITRO EXAMINATION OF IN VIVO SYSTEMS

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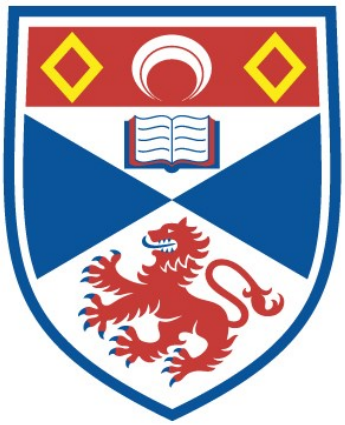
A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



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Thermodynamics of the complexation between Asparagine and first row Transition Metal Ions.

An *in vitro* examination of *in vivo* systems.

A Thesis for the Degree of Doctor of Philosophy,

at the University of St. Andrews,

by Allan Cameron Baxter, B.Sc. (St. Andrews).

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Abstract

Thermodynamic parameters have been obtained for the complexes formed between protons, first Transition Series metal ions and the asparaginate ion in aqueous solution at 25°C in an ionic background of 3.00 M (sodium) perchlorate. The metal ions under consideration are manganese(II), iron(II), cobalt(II), nickel(II), copper(II) and zinc(II).

The techniques employed were potentiometry to obtain the formation constants (and hence ΔG°) for the complexes, and aqueous titration calorimetry to obtain the corresponding ΔH° (and hence ΔS°) values.

The results show that iron, cobalt, nickel and zinc are capable of binding up to three asparaginate ligands, whereas for manganese and copper the maximum number of bound ligands is two. All the complexes reported one simple A_nB species: no hydroxy, protonated or polynuclear species were detected.

The results are used(1) to discuss an unusual "homologue" effect whereby the trends among homologues for bonding to protons and to metal ions are opposite to each other, this apparently being a repercussion of solvation differences between homologues and (2) to show that quite a large proportion of asparaginate in

blood plasma may be complexed to Zn(II), Fe(II), and Co(II), as well as the expected Cu(II).

The techniques of potentiometry and calorimetry are further employed to determine the thermodynamic parameters for three ternary systems, one, copper(II)-histidinate-threoninate, which is known to exist *in vivo*, and two others, copper(II)-asparaginate-histidinate and copper(II)-asparaginate-threoninate, which have not yet been detected. Structures are suggested for the complexes Cu.asn.his., Cu.asn.his.H^+, Cu.asn.thr., Cu.his.thr. and Cu.his.thr.H^+. In these complexes histidinate is tridentate to Cu(II), threoninate is bidentate and asparaginate is intermediate between bi and tridentate. The site of proton attachment in the protonated complexes is the primary amine site of the histidinate ligand.

Throughout the work extensive use was made of computer programs, all of which are described in the chapter entitled "Computational Aspects".

A.C. Baxter
8.11.76

Declaration

I declare that this thesis is my own composition, that it is a record of the work carried out by me, and that the work has previously been submitted in an unsuccessful application for a higher degree to this University on 4 August 1975.

The work was carried out in the Chemistry Department, University of St. Andrews, between September 1971 and October 1974.

A.C. Baxter
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Certificate

I hereby certify that Allan Cameron Baxter has researched under my supervision and has fulfilled the conditions of Ordnance General Number 12 and Resolution of the University Court, 1967, Number 1 and is qualified to resubmit this thesis in application for the Degree of Doctor of Philosophy.

David R. Williams,
Department of Chemistry,
University of St. Andrews.

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A.C. Baxter
2.11.76

NOMENCLATURE

The following is a list of symbols and abbreviations which have been used throughout this work.

A and A' - Total concentrations of ligands A and A'

a and a' - Free concentrations of ligands A and A'

B and B' - Total concentrations of central groups (metals) B and B'

b and b' - Free concentrations of central groups (metals) B and B'

E - Electromotive force (e.m.f.)

E^o - Standard e.m.f. for a glass//calomel electrode pair

F - Faraday

f - Activity coefficient

H - Total hydrogen ion concentration

h - Free hydrogen ion concentration

I - Ionic strength

i - Electrical current

J - Joule (unit of energy - usually appears as kJ = 10^3 joules)

K - Kelvin (unit of temperature)

k_{a_n} - Stepwise concentration formation constant for a protonation reaction

k_{M^n} - Stepwise concentration formation constant for a complexation reaction - $mol.dm^{-3}$

k_w - Ionic product of water (also wk)

p and p' - Number of ligands A and A' in a complex

q and q' - Number of central groups (metals)B and B' in a complex

r - Number of protons H in a complex

s - Standard deviation

V - Voltage (often appears as mV = 10^{-3} volts)

$\overline{Z}$ - Average Number of ligands A bound per central group (metal) B

β^{o}_{pqr} - Thermodynamic overall formation constant for complex $A_pB_qH_r$

β_{pqr} - Concentration overall formation constant for complex $A_pB_qH_r$

ΔG^{o} - Standard Gibbs free energy change

ΔH^{o} - Standard enthalpy change

ΔS^{o} - Standard entropy change

} with 3.00 M $(Na)ClO_4$ as standard solvent

ϵ - Extinction coefficient

ν - Frequency (in cm^{-1})

Ω - Electrical resistance (ohms)

The abbreviations for the amino-acids (asn, his etc.) are as according to Specialist Periodical Reports : Amino-acids, Peptides and Proteins, Volume 4, Chapter 5, Chemical Society, London, 1972.

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CHAPTER 1 INTRODUCTION

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CHAPTER 1 INTRODUCTION

The acquisition of knowledge about living systems has progressed remarkably in the last few years. New techniques have played a large part in these advances, and co-ordination chemistry, bio-inorganic chemistry, organo-metallic chemistry, enzymology and molecular biology have together made a significant contribution.

During the 1930's the theoretical side of chemistry developed greatly, culminating in Pauling's work entitled "The Nature of the Chemical Bond"[1]. Hedges noted that "inorganic chemistry was again coming into its own"[2], a comment which is again relevant today, but this new revival of inorganic chemistry is due to expansion in both the theoretical and the practical fields. New experimental techniques have been discovered and developed with the aid of more efficient instruments, and the introduction of high-speed computers, which has had a profound effect on science and technology in general, has benefitted chemistry in particular.

Chemistry has been traditionally divided into inorganic, organic and physical chemistry, but these divisions have always been regarded as over-simplifications, and today are becoming increasingly ill-defined. This is shown by the vast field of organo-metallic chemistry, and by the fact that entire journals are devoted to physical-organic chemistry. Hybrid names have been produced to define the new areas of research, such as organo-metallic chemistry and bio-inorganic chemistry.

The origin of bio-inorganic chemistry.

The term bio-inorganic chemistry, or inorganic biochemistry, was

used by Williams[3] to describe the studies of metal compounds in biological systems and the use of metal co-ordination compounds as models of metallo-enzymes. Bio-inorganic chemistry had its beginnings just after the second world war, when chemists began to study complexation reactions between metal ions and biologically important simple ligands such as amino-acids. Within the last 10-15 years rapid advances have been made in the development of bio-inorganic chemistry, and the interest that inorganic chemists and biochemists now have in each others' fields has been given a considerable boost by the employment of such techniques as X-ray crystallography in the study of metallo-proteins.

The inorganic chemist's interest in the concepts of complex stabilisation by ligand-field effects, hard and soft acid-base theory, catalysis by metal complexes, the effects of complexation on e.m.f., and general thermodynamic and kinetic effects in complexation is leading towards an explanation of the behaviour of metallo-enzymes[4].

Many research groups are studying the formation and stability of biological metal ion-containing compounds of a wide range of molecular weights. Many other groups are devoted to the design of drugs as therapeuticals, for example, anti-cancer drugs. Such drugs often contain a metal ion, and some are metal complexes of already established drugs.

Williams has pointed out that life is as much inorganic as organic[5], so it is pleasing to record that biochemistry, which has received so much attention from organic chemists, is having its inorganic aspects rapidly developed.

The importance of metals in human metabolism.

The importance of metals in human metabolism was realised as long ago as 1500 BC when Prince Iphyclus was cured of impotence by a suspension of rust in wine[6].

Of the 87 naturally occurring elements, eighteen can be considered as being essential to the human body. Eight of these, H, C, N, O, P, S, Cl and I, are non-metals and ten, Na, K, Mg, Ca, Mn, Fe, Co, Cu, Zn and Mo, are metals.

The first four non-metals, H, C, N and O, form amino-acids, simple organic molecules, which can build up into long chains in peptides and proteins. Sulphur is also contained in the amino-acids cysteine (S-H group), cystine (C-S-S-C) and methionine ($S-CH_3$). The non-metals H, C, N and O also form carbohydrates, steroids, lipids and nucleic acids. Phosphorus is contained in nucleic acids and in certain classes of lipid, and along with sulphur and chlorine, is also essential as ions (PO_4^{3-}, SO_4^{2-} and Cl^-) in maintaining electroneutrality, osmotic pressure and cell volumes. Iodine occurs in hormones such as thyroxine.

The metal ions can be divided into two subgroups, the bulk metal ions sodium, potassium, magnesium and calcium, charge carriers and aquated cations, and the last two are also of prime importance in the action of adenosine triphosphate (ATP), and the trace metal ions Mn(II), Fe(II)/(III), Co(II)/(III), Cu(II), Zn(II) and Mo(VI).

Another seven elements, Si, V, Cr, Se, Sn, Br and F, have become essential over the years, apparently by following the scheme poisons $\longrightarrow$

tolerable impurities → useful elements → essential elements[7]. Dietary deficiencies of these elements produce animal growth rates as low as two-thirds of the normal growth rates. It appears that boron, germanium and nickel have now started along the trail to becoming essential elements, and in recent years cadmium, mercury and lead have become important through pollution of the environment by civilization.

The divalent trace metal ions Mn(II), Fe(II), Co(II), Cu(II) and Zn(II), were chosen as part of the subject matter of the present work, and Ni(II) was included to complete the $3d^{5-10}$ series. The work is a laboratory investigation of possible in vivo reactions of metal ions and at this point it is appropriate to consider the biological rôles of these metal ions.

The biological rôles of the first row transition metal ions.

Manganese.

Manganese salts are poorly absorbed from the intestine, but after parenteral administration manganese is concentrated in the liver and kidneys, and excreted largely into the colon and bile, with only a small portion in the urine. Ingestion of excessive quantities of manganese appears to interfere with absorption of iron, thus causing an anaemia which is readily prevented by increasing the dietary iron.

Manganese is intimately bound to arginase of liver and is required for the action of many enzymes which perform redox reactions, e.g. the oxidative β-ketodecarboxylases, certain peptidases and muscle adenosine triphosphatase.

Mn(II) can replace Mg(II) in the DNA scheme but the major reactions give a different range of daughter products.

(ii) Iron

Nature has provided iron with a stereochemistry in vivo, which, with one exception (see later), cannot be reproduced in the laboratory.

65-70% of body iron is found in haemoglobin. The metal ion co-ordinates octahedrally but one of the two axial sites reversibly binds and releases molecular oxygen. The other axial site is occupied by the imidazole nitrogen of a histidinate residue, while the four equatorial sites are occupied by the donor nitrogen atoms of a porphyrin ring. The structures of cytochrome C and peroxidase are similar, but in the former histidinate residues occupy both axial sites. Cytochrome C is an electron-transferring protein and is intimately united with the interconversion between the ferrous and ferric state of the haem iron[8]. In peroxidase one of the axial sites is used for binding hydrogen peroxide, and there is some evidence that the central iron atom is oxidised to the (IV) state[9].

In his studies on haemoglobin-type molecules Wang successfully prepared a macromolecule which reversibly complexed molecular oxygen[10]. This molecule contained 1-(2-phenylethyl)-imidazolecarbonmonoxyhaem diethyl ester embedded in a matrix of an amorphous mixture of polystyrene and 1-(2-phenylethyl)-imidazole, and showed a carbonmonoxyhaem-like absorption spectrum. On removal of the bound carbon monoxide, a haemoglobin-like absorption spectrum was obtained. The molecule was shown to be stable in presence of water.

(ii) Cobalt

Cobalt is best known for being the central ion in vitamin B_{12}, where it is in the (III) state[11]. The central part of the structure of the co-enzyme form of vitamin B_{12}, cobamide, bears some resemblence to the iron-porphyrin systems. Vitamin B_{12} itself, cyanocobalamin, is needed in the body to form haemoglobin, and deficiencies cause pernicious anaemia.

Cobalt (II) complexes are carriers of molecular oxygen, and also in the (II) state cobalt is associated with low symmetry sites in enzymes.

The zinc atoms in carboxypeptidase have been replaced in vitro by cobalt, with a resultant increase in peptidase activity[12].

(iv) Nickel

As previously mentioned, nickel has started along the evolutionary trail towards becoming an essential element, and the effects of introducing nickel compounds into the body have been studied. The monoxide and the tetracarbonyl are among the nickel compounds known to cause cancer, but on the other hand certain nickel dialkyldithiophosphates, along with corresponding platinum and palladium compounds, have been found to have anticancer activity[13].

(v) Copper

Human plasma, or serum, is approximately 18 μM in copper, 90-95% of which is tightly bound to an alpha-2-globulin, ceruloplasmin[14]. This is a true metallo-protein and so the eight atoms of copper bound to it are not exchangeable under physiological conditions, either in vivo or in vitro.

For this reason analogies cannot be drawn between ceruloplasmin and transferrin, because ceruloplasmin is not a transport protein for copper in the same sense as transferrin functions for iron.

The remaining 5–10% of the copper is more loosely bound to albumin. Albumin-copper is not a metallo-protein, for the copper bound to albumin is in equilibrium with ionic copper in solution. There is evidence to suggest that copper initially binds to albumin on introduction into the bloodstream[15].

Copper deficiency has been observed in infants receiving only milk, and the prime manifestation is a microcytic, normochromic anaemia. This condition responds to administration of ceruloplasmin or inorganic copper. Excessive tissue deposits of copper are seen in Wilson's Disease, which responds to treatment with D-penicillamine.

Known metabolic functions of copper relate to its presence in tyrosinase, uricase and perhaps cytochrome oxidase. Erythrocuprein, a copper containing protein of uncertain function, occurs in human erythrocytes.

(vi) Zinc.

Although zinc is widely distributed in foodstuffs, deficiency has been reported[16]. Many years ago zinc deficiency is believed to have led to dwarfism among some central European peoples, and deficiency still exists today in certain parts of the world.

Zinc helps to control the physiological pH by hydrolysis and is one of the constituentsof enzymes such as carboxypeptidase, carbonic anhydrase and alcohol and lactate dehydrogenases.

The aqueous solution chemistry of these metals will be discussed in Chapter 8.

Reasons for studying metal-amino-acid complexes.

There is significant evidence that amino-acid complexes are a fruitful research area. Of the five main areas of cancer chemotherapy, two often involve amino-acids. They are (i) the antimetabolite approach, which functions because tumours mistakenly construct new cells, not from metabolites, but from administered chemicals which are very similar in structure to the cancer cell's usual metabolites, and (ii) the enzyme treatment of cancers which uses glutaminase and asparaginase, and removes an essential metabolite en route to the cancer[17-18].

The other three areas of cancer chemotherapy are (i) alkylating agents, (ii) hormone therapy and (iii) synergistic combinations of established treatments.

Livingstone et al investigated metal chelates of DL-methionine and ethionine and attributed their activity to that of the ligand rendering the metal ions fat-soluble and thus capable of reacting inside the cell[13]. Amino-acids have been used to direct nitrogen mustards into cancers, e.g., phenylalanine mustard which is used to control malignant myeloma[19] and Burkett's Lymphoma[20]. Over a period of time tumour cells recognise anti-cancer therapeuticals and build up a resistance to them, but this is less likely to happen when part of the drug is an amino-acid which tumour cells require in order to actively reproduce. Some cancer cells cannot

synthesise all the amino-acids synthesised by normal cells, e.g. lymphatic leukaemia cells require an external source of asparagine. Intercepting and removing this amino-acid from the bloodstream by treatment with L-asparaginase is a well-known approach to exploiting the difference between normal and cancer cells and so starving the abnormal cells of an essential metabolite. A parallel situation occurs with the treatment of myoblastic leukaemia with L-glutaminase[17]. Competitive metal complexing has been suggested as an alternative and possibly better method of removing these amino-acids from the bloodstream[21].

Sarkar et al have shown[14] that besides the copper bound to ceruloplasmin and albumin, there is a third very small but physiologically important fraction of copper in serum bound to amino-acids. This will be discussed in the next section, and is another good reason for studying amino-acid complexes with metal ions.

The discovery of ternary complexes in vivo

As previously mentioned, copper on introduction into the bloodstream appears to bind to albumin. However Sarkar et al discovered that low molecular weight ligands existed that could compete with albumin for the binding of copper ions[14]. Further experiments revealed that these ligands were amino-acids, especially histidine and threonine. This work led to the discovery of the first copper containing ternary complex in vivo, Cu(II)-histidinate-threoninate (Cu(II).his.thr)[22]. More recent work has furnished evidence for compounds intermediate between the copper-albumin complex

and the simple binary species Cu(II).his.$^{+}$ and Cu(II).thr.$^{+}$ These are also ternary complexes, Cu(II).albumin.his and Cu(II).albumin.thr. respectively[23]. The albumin in these complexes is bound to copper through the N-terminal aspartate residue of the protein[24]. Other ternary complexes discovered include Cu(II)–histidinate-glutaminate (Cu(II).his.gln.) and Cu(II)–histidinate-serinate (Cu(II).his.ser.)[25].

Binary and ternary copper(II) complexes are therefore known in vivo. The study of the thermodynamics of formation for the binary complexes should not be an end in itself, but the results obtained from these systems should be used to assist in the determination of thermodynamic parameters for ternary systems under the same conditions, and then to predict the structures of the ternary species.

Selection of an amino-acid.

Previous studies on metal amino-acid complexation in this laboratory have included the 3d[5-10] metal ions with histidinate[26-27], tryptophanate[26] and phenylalaninate[28], while glutaminate and serinate[29] were examined shortly after the present work was commenced.

Examination of the literature revealed that asparaginate complexation had received less attention than might be expected for such a biologically important ligand, and in some cases the results from various workers disagreed. For example, Tanford and Shore[30] reported a Co(II).asn$_3^-$ complex, but Ritsma et al[31] detected only Co(II).asn$^+$ and Co(II).asn$_2$. In view of this situation it was decided to undertake an investigation of the complexation of asparaginate with the ions Mn(II), Fe(II), Co(II), Ni(II),

Cu(II) and Zn(II). Asparaginate is an interesting ligand in that it has an amide group which may bind to the metal ion.

The logical extension of the work on binary asparaginate complexes was to examine some ternary systems involving asparaginate under the same conditions. Although ternary complexes between Cu(II), asparaginate and another amino-acid have not yet been detected in vivo this does not mean that such complexes do not exist. However, the concentration of asparaginate in human serum (44 μM) is considerably lower than either histidinate (81 μM) or threoninate (117 μM) and so ternary species such as Cu(II).asn.his. and Cu(II).asn.thr. will be formed in low concentrations.

Three systems, Cu(II).asn.thr., Cu(II).asn.his. and Cu(II).his.thr., were examined under the same conditions as the binary systems and it was hoped to be able to deduce the structures of the complexes, both from the results for the binary systems and from the known complexing tendencies of metal ion and competing ligands.

Asparagine in vivo.

The amino-acid asparagine is non-essential in the human diet, and in the body is produced from aspartate, which in turn arises from trans-amination between glutamate and oxaloacetate.

$$\underset{\text{glutamate}}{{}^{-}O_2C.CH_2.CH_2.CH(NH_3^{+}).CO_2^{-}} + \underset{\text{oxaloacetate}}{{}^{-}O_2C.CH_2.\overset{O}{\overset{\|}{C}}.CO_2^{-}}$$

$$\underset{\alpha\text{-ketoglutarate}}{{}^{-}O_2C.CH_2.CH_2.\overset{O}{\overset{\|}{C}}.CO_2^{-}} + \underset{\text{aspartate}}{{}^{-}O_2C.CH_2.CH(NH_3^{+}).CO_2^{-}}$$

(At physiological pH, 7.4, amino-acids exist as zwitterions, and carboxylic acids are deprotonated, therefore it is correct to write these compounds as shown overleaf).

From aspartate there are two possible routes to asparaginate. In some organisms it is formed by reaction of ammonia, aspartate and ATP, and in others the amide nitrogen of glutaminate is transferred to aspartate.

(i) $^{-}O_2C.CH_2.CH(NH_3^{+}).CO_2^{-}$ + NH_3 + ATP $\longrightarrow$

aspartate

$H_2NC(=O).CH_2.CH(NH_3^{+}).CO_2^{-}$ + inorganic phosphate

asparaginate

(ii) $^{-}O_2C.CH_2.CH(NH_3^{+}).CO_2^{-}$ + $H_2NC(=O).CH_2.CH_2.CH(NH_3^{+}).CO_2^{-}$ $\longrightarrow$

aspartate glutaminate

$^{-}O_2C.CH_2.CH_2.CH(NH_3^{+}).CO_2^{-}$ + $H_2NC(=O).CH_2.CH(NH_3^{+}).CO_2^{-}$

glutamate asparaginate

Asparaginate ultimately enters the citric acid cycle in the form of oxaloacetate. The first step is hydrolysis by the enzyme asparaginase to aspartate and ammonia. The resultant aspartate undergoes transamination with α-ketoglutarate to form oxaloacetate.

asparaginate $\xrightarrow[\text{hydrolysis}]{\text{asparaginase}}$ aspartate + NH_3

aspartate + α-ketoglutarate $\longrightarrow$ glutamate + oxaloacetate.

Alternatively, in plants, aspartate undergoes direct elimination of ammonia to yield fumarate. This reaction is catalysed by the enzyme aspartase, which is not present in animal tissues.

$$^{-}O_2C.CH_2.CH(NH_3^{+}).CO_2^{-} \xrightarrow{\text{aspartase}} O_2^{-}C.CH{:}CH.CO_2^{-}$$

aspartate — fumarate

The HSAB approach to metal-ligand bonding.

The strengths of metal-ligand bonds are conveniently systematized using the theory of Hard and Soft Acids and Bases[32-37]. This approach assumes that all bonds between heteroatoms may be considered as having an acid and a base part. This acidity or basicity is decided by the number of valence electrons associated with a species and the ease with which they can be rearranged. Properties employed in classifying a species as hard or soft, acid or base, are summarised in Table 1[38].

The main principle behind HSAB theory is that strong bonds are formed only between hard acids and hard bases or soft acids and soft bases. Hard-soft bonds are known in the solid state (e.g. Al_2S_3), but in solution they are either very weak or do not exist (e.g. Al_2S_3 hydrolyses to $Al(OH)_3$ in solution).

In practice it is found that the hard acid metal ions prefer ligand donor atoms of the first short period.

$N \gg P > As > Sb$

$O \gg S \simeq Se > Te$

$F > Cl > Br > I$

On the other hand, soft acid metal ions prefer

$N \ll P > As > Sb$

$O \ll S < Se \simeq Te$

$F \ll Cl < Br < I$

Such observations not only explain the distribution of metals on the earth's surface, e.g. Na(I), K(I), Mg(II), Ca(II) and Al(III) are found as ores of hard bases, such as oxides and carbonates, and Cu(II), Hg(II) and Pb(II) are found as ores of soft bases such as sulphides, but also the distribution of bonds in vivo where Na(I), K(I), Mg(II) and Ca(II) exist as aquated cations, water being a hard base, and the transition metals show a clear trend from oxygen donors through to sulphur donors as hardness decreases.

The metal ions under consideration in the present work are Mn(II), Fe(II), Co(II), Ni(II) Cu(II) and Zn(II). Of these, only Mn(II) is hard and the rest are borderline between hard and soft. In vivo and in vitro metal ions are often rendered hard or soft by their environment. In carbonic anhydrase zinc binds $I^- > Br^- > Cl^- > F^-$, i.e. in the enzyme environment (sulphur and nitrogen donors from amino-acids) zinc is acting as a soft acid. However, in aqueous solution the order of binding is reversed, i.e. the hard solvation sphere has rendered Zn(II) hard. This phenomenon, whereby hard (or soft) bases are attracted to an acid which is rendered hard (or soft) by its environment, is known as symbiosis, which means, literally, "living together for mutual benefit"[38].

Metal ions in biological systems are often in a state of suspension between two oxidation states. The lower state can be stabilised by addition of soft ligands and the higher oxidation state by hard ligands. However, if very hard or very soft ligands are added, the metal ion becomes anchored in one oxidation state, and the living process (e.g. a redox reaction) does not occur. This is an example of poisoning and the best-known poisons are

usually acids or bases that are so strongly held to the active sites of an enzyme that the sites are blocked off. Examples of soft acid poisons are cadmium ions and methylmercury, and of soft base poisons are carbon monoxide, cyanide ions and sulphide ions.

HSAB principles can be (and have been) used in the design of therapeuticals to remove unwanted metal ions. The earliest therapeuticals were known before HSAB but it can be seen that the donor groups and metal ions removed are in agreement with HSAB principles.

(a) Desferrioxamine B (discovered 1960) contains several oxygen donor atoms and has been used to remove excess Fe(III). The HSAB classification of this therapeutical is hard.

(b) EDTA (discovered 1935) has four oxygen and two nitrogen donor atoms, and, administered as $Na_2[Ca\,EDTA]$, has been used to remove Co(III) and Pb(II). The HSAB classification of EDTA is borderline.

(c) D-penicillamine has one S, one N and one O available, and removes copper in either of its common oxidation states (e.g. in Wilson's disease) and is classified on the soft side of borderline.

(d) BAL (British anti-lewisite), or 2,3,dimercaptopropanol, has two sulphur atoms which help to remove arsenic compounds, gold, and mercurous and mercuric mercury. Its HSAB classification is soft.

In recent years HSAB principles have been used in assisting in the design of anti-cancer drugs. Rosenberg has reviewed a large number of Pt(II) and Pt(IV) complexes which have been found to exhibit anti-tumour

activity[39]. The mechanism through which these complexes exhibit this effect has not been firmly established, but it is thought that the site of their action is inside the cancer cells at the nuckar DNA, and that the complexes selectively inhibit the synthesis of new DNA polymers, but do not react with RNA and protein. The complexes investigated contained two cis Cl groups and it is suggested that these are lost to form a purine-Pt-purine crosslink between nitrogens of neighbouring purines. Platinum is one of the metals in the Ahrland and Chatt triangle based on group VIIIb of the transition series[40]. Although Pt is the most widely known member of this group as far as pharmacological properties are concerned, Rh and Ir also have a considerable anti-bacterial history[41]. Further, as we move away from the soft elements of the triangle, it is possible, now that scales of softness are available, to judiciously select ligands so they symbiotically render the metal's bonds to be as soft as those of a member of the triangle, e.g. Mo(cyclopentadienyl)$_2$Cl$_2$ has a metal which is as soft as Pt(NH$_3$)$_2$Cl$_2$ and so both react similarly with amino-acids etc.

Although HSAB principle are useful in designing therapeuticals, they must be used in conjunction with other principles, such as stereo-chemistry, solubility and polarisability.

Table 1 Classification of Hard and Soft Acids and Bases

PROPERTY	Hard Acid	Soft Acid	Hard Base	Soft Base
Polarisability	Low	High	Low	High
Electro positivity or negativity	High	Low	High	Low
Positive or negative charge	Large	Small	Large	Small
Types of bond usually associated with acid or base	Ionic, electrostatic	Covalent, π	Ionic, electrostatic	Covalent, π
Outer electrons on donor atoms	Few, not easily excited	Several, easily excited		
Available empty orbitals on donor atoms			High energy, Inaccessible	Low lying, accessible

Some metal ion-dependent reactions.

While kinetics and stereochemistry play a large part in biological systems, thermodynamics also has its place, as is shown by the importance of adenosine triphosphate (ATP) and the frequency with which it takes part in biological reactions. The widely-held view of the mode of action of ATP, that hydrolysis of one phosphate group provides a large amount of energy to drive other reactions, has received much discussion in recent years[42-44], but the possibility of catalysis by magnesium ions was omitted from the discussion.

A "break-through" in the understanding of the action of "aspirin" has been reported[45], but the possible significance of metal ion involvement was not considered until recently. The understanding of the role of "aspirin" is that it inhibits the formation of prostaglandins,but in 1954 Schubert suggested[46] that the role of "aspirin" was to "remove or inactivate copper present in an intracellular site".

Sorenson[47] discovered that anti-inflammatory agents (drugs used to treat the pain and joint inflammation of arthritis) were more active in the form of their copper complexes. "Aspirin", for example was 20 times as active in its copper complex than as the free ligand in some model systems.

On the results of ulcerogenic activity experiments, Sorenson postulated that unchelated inflammatory agents removed copper from stomach tissues to form the active copper chelates. The connective tissue of the stomach needs copper for tissue synthesis and its absence could lead to ulcerative lesion. He also discovered that the copper "aspirin" complex

was as effective as "aspirin" alone in depressing pro-inflammatory prostaglandin synthesis[47].

Methods used in the study of metal-ligand complexes.

The methods currently available for studying the formation, electronic structure and three-dimensional structure of metal-ligand complexes are numerous, and a brief description of some of the more important ones is given below. A longer description of potentiometry and calorimetry, the two techniques used in this work, follows at the end of this section.

Spectroscopic techniques

(a) Mössbauer Spectroscopy

The use of this nuclear gamma-ray resonance spectroscopy is limited to species containing the $^{57}_{28}Fe$ nucleus and to samples in the solid state, but due to the wide distribution of iron in biological systems this is no problem as Mössbauer has several advantages over other forms of resonance spectroscopy. There are no interfering signals from other atomic species, and the Mössbauer nucleus does not disturb the electronic environment being studied. Haemoproteins, haem prosthetic groups and iron-sulphur proteins have been studied by Mössbauer[48-49], which, along with epr (see below) affords an opportunity to determine the detailed electronic configurations, a necessary step towards the chemical basis of protein function.

(b) Nuclear Magnetic Resonance.

This technique can be applied to any compound containing a nucleus with a spin, e.g. $^{11}_{5}B$, $^{19}_{9}F$ and $^{31}_{15}P$, and more especially hydrogen, the

simplest nucleus, and the technique yields volumes of information concerning the structure of any species containing this nucleus, e.g. the substitution pattern of an aromatic ring, whether a carbon-carbon double bond is cis or trans etc. Both complex molecules[50] and simple molecules of biological importance[51] have been studied by nmr.

(c) Nuclear Quadruple Resonance.

For a nucleus to show an nqr signal it must have a nuclear spin of one or greater. The main nuclei studied have been the halogens, except $^{19}_{9}F$ which has no nqr, and $^{59}_{27}Co$[52]. Information concerning the bonding in transition metal complexes can be obtained using this technique.

(d) Electron paramagnetic resonance.

Ions with unpaired electrons give an epr signal, hence it is possible to study Cu(II), Fe(III), Co(II) and Mo(V) using this technique. The mechanism of the reaction between molybdenum and riboflavin was elucidated using epr and its importance was emphasised[53].

(e) Crystallography.

Of the three main branches of crystallography, that using X-rays is by far the most important, and by comparison the more precise neutron and electron diffraction crystallographies play only a minor part. The elucidation of the structures of complex molecules, such as vitamin B_{12}[54], has played a great part in the advances made in the biological sciences in the last 25-30 years.

(f) Optical Rotatory Dispersion and Circular Dichroism.

These two techniques are used with optically active complexes, and

involve a degree of preparative difficulty. They have been applied successfully to complexes of transition metals and amino-acids[55], and yield information concerning the configuration of the complexes.

(g) Atomic Emission Spectroscopy.

Large scale surveys of metal content of soils and plant materials have been carried out using this technique, where electric arcs or sparks are used to produce emission spectra of the various atoms in the sample. By linking the read-out systems to a computer it is possible to obtain a print-out for over 20 elements by spectrographic analysis of a small sample of dried soil or plant material, and in this way deficiencies or excesses of elements which may affect the well-being of plants and animals may be detected. Human tissue studies have also been carried out using this technique[56].

(h) Atomic Absorption Spectroscopy.

This technique involves the absorption of light by free atoms in a suitable vapour phase or atom cell, which is produced either by a flame or by an electrically heated graphite furnace or carbon rod. Like atomic emission spectroscopy this technique has been used to analyse metals in biological samples, and has also proved successful in forensic science[57].

(i) Infra-red Spectroscopy.

This technique reveals which donor atoms in a ligand are involved in complexation to a metal ion, which potentiometry and calorimetry cannot decide unambiguously. For example, Carlson and Brown[58] used i.r. spectroscopy to study the distribution of transition metal-histidinate complexes,

and also complexes with imidazole and alaninate, using deuterium oxide as solvent. They were able to assign absorption wavelengths to different bonds by studying the IR spectra of a series of deuterated derivatives of these ligands.

(j) Ultra-violet and visible spectroscopy.

This technique reveals the electronic and conformational changes involved when complexation takes place. These exhibit themselves in the peak height and the position of the peak. The technique has been used to obtain formation constants for a number of systems, such as iron (II)-phenanthroline[59] and copper(II)-ammonia[60].

Kinetic studies.

Biochemical reactions are often very fast and specific. In inorganic chemistry it is uncommon for a ligand to complex with a metal ion with such a specificity as an enzyme combines with its substrate.

The problem of fast reactions led to the development of stopped-flow techniques and later to relaxation methods such as temperature and pressure-jump to study bio-inorganic reactions. The most recent work includes stopped-flow studies on redox systems involving such metal ions as Co(III), Fe(III) and Ce(IV) with biologically important ligands such as hydroxy - and thio-carboxylic acids[61-62]. In many of these reactions weak complexes are formed prior to the reduction of the metal ion and so a conventional potentiometric investigation of the formation of these complexes is not possible.

Potentiometry.

This was one of the two methods used to study the metal-ligand systems in the present work. It is a well-established technique, and is by far the most accurate and widely applicable technique currently available for the study of ionic equilibria.

Measurements of the potentials of galvanaic cells were used to determine the activities of metal and hydrogen ions at the end of the last century[63], and were employed in equilibrium studies of a number of metal and proton complexes in the years leading up to the first world war[64]. The potentiometric method has since been used extensively in many branches of solution chemistry, and many different kinds of electrodes have been designed.

Some of the earliest work used metal electrodes, in either wire, stick or sheet form, dipping into a solution of the same metal ion[65]. Complications arose with multivalent metal ions, when the solution of metal ions could be reduced to ions of lower charge. However,the couples Cu^{2+}/Cu, Au^{3+}/Au and In^{3+}/In were satisfactory because of the low equilibrium constants for the reduction reactions.

Equilibrium between electrode and solution is often quicker if the pure metal is replaced by an amalgam, which is conveniently prepared by dissolving the metal in pure mercury or by electrolysing a solution of the metal salt, using a mercury cathode. Air must be carefully excluded since oxidation often occurs. Half-cells of the type B^{2+}/B-Hg have been used to study complexes of a number of metal ions, and among the recent investigations

of bio-inorganic interest is the work of Williams on copper-histidinate complexes[27].

Another type of electrode sometimes used is the quinhydrone electrode, a pre-war invention. In spite of a number of disadvantages, such as decomposition in alkaline solution and reduction by certain metal ions, the quinhydrone electrode has been used for a number of careful studies of solution equilibria[66]. Quinhydrone is an equimolecular compound of benzoquinone and hydroquinone and in contact with an electrode of gold or platinum, and with hydrogen ions in a constant ionic medium, rapidly acquires a potential. It may be more precise than the glass electrode, which, however, is more widely used.

The glass electrode determines the hydrogen ion activity in solution, so that a reaction dependent upon hydrogen ions may be followed in solution. Ligands which complex with metal ions also associate with protons in solution, and so the complex reaction, being competitive, is pH dependent. The electrode process does not involve electron transfer; so glass electrodes can be used in the presence of oxidising or reducing agents or of any substance which does not attack or adhere to the active surface. With careful handling glass electrodes are easy to use and come to equilibrium extremely rapidly. For these reasons the glass electrode was used in the present work.

The original "glass cell" was described by Haber in 1909[67], and was the subject of a review by Hughes[68]. The earliest results were mostly confined to metal-inorganic ligand reactions[69] and it was not until the 1940's that the glass electrode became widely used in determining the stability

constants for complexes between metal ions and organic ligands. In the early 1950's Albert and Perkins used the technique to determine stability constants for complexes between metal ions and amino-acids[70-71], and since then the range of metal ions and ligands has widened enormously.

The calomel electrode, $Cl^-/Hg_2Cl_2(s)/Hg(l)$ is most commonly used as a reference half cell, since commercial pH meters are designed for use with a cell consisting of a glass electrode and a calomel electrode immersed in potassium (sometimes sodium) chloride. It has also been used in conjunction with metal and amalgam electrodes. Another reference system is the half-cell Ag^+/Ag which has been used by various workers in metal ion complexation studies[72].

Other ion-sensitive electrodes are now available[73], but as yet they do not have the applicability, stability and sensitivity of the glass electrode. The most widely used of these new electrodes are the fluoride and calcium electrodes, and amino-acid electrodes[74] are now becoming more popular.

Calorimetry.

This technique gives, directly, the enthalpy of formation, i.e. the strength of a metal-substrate bond being formed in solution. Together, potentiometry and calorimetry give the standard Gibbs free energy, ΔG^o, the standard entropy, ΔS^o, and the standard enthalpy, ΔH^o, changes involved in the formation of a complex.

Although inorganic chemists have a large number of methods to measure the size, shape and three-dimensional structure of their molecules

they have largely ignored calorimetry as a means of determining the bond strengths. On the other hand biochemists have readily accepted calorimetry, which, although still in its infancy is being developed to such a degree of sophistication that it could tackle the complex energistics of man[75].

In general each calorimetric investigation involves the matching of an instrument to the chemical system and so large numbers of calorimeters are in existence. In its simplest form a calorimeter is no more than a reaction vessel, in which known amounts of reagents can be mixed, fitted with some device whereby the resulting temperature change can be measured. Many types of reaction calorimeter have been proposed for different purposes, and the precision with which the heat change can be measured is dependent mainly upon the care with which the calorimeter vessel itself, its environment and the device for mixing the reagents have been designed.

There are two methods by which the reagents may be mixed. In both cases one reagent is already positioned in the calorimeter, and the other reagent is added either by breaking an ampoule containing it, or by adding it from a burette. The first method is useful when the second reagent is unstable in air, and the ampoule can be left immersed in the calorimeter vessel until temperature equilibrium is obtained. The ampoule is then broken, usually by the stirrer[76].

This method has the disadvantage that only one addition is possible for each experiment. To repeat the experiment, or to change the ratio of reactants, the apparatus must be reassembled. Consequently, the second method, addition of reagent from a burette, is preferred. Danielsson et al

have set up a Metrohm piston burette to deliver titrant through a glass spiral, immersed in the calorimeter bath, and hence into the calorimeter itself, emerging through a capillary burette end below the surface of the solution in the calorimeter[77]. Their approach is followed in the present work (see Chapter 6).

Many calorimeters are described in the literature[78], and some of these instruments have been used to measure enthalpies of formation for complexes of biologically important ligands with metal ions[79]. The technique has many advantages over existing biochemical techniques, one of them being that translucent solutions, necessary for spectrophotometry, are not required. Biological calorimetry has therefore become established as a new and important discipline.

Choice of Method.

The examination of natural systems produces difficulties, such as insolubility, arising from complexes involving high molecular weight proteins, so that a wide range of techniques must be used. The alternative approach is to study complexation between metal ions and biochemically important simple ligands such as amino-acids, and extrapolate the results to apply to systems in vivo. This is the approach used by Perrin et al in Canberra, where large quantities of potentiometric data on simple systems have been collected and combined, using computer models, to produce important biological conclusions. This approach is used in the present work where complexation between asparaginate and manganese(II), iron(II), cobalt(II), nickel(II), copper(II) and zinc(II) and protons is examined using

potentiometry and calorimetry, and the same techniques are used again to determine thermodynamic parameters for one known and two possible in vivo ternary systems. For aqueous complex studies these two techniques are the most productive in terms of conclusions yielded per given amount of data and time, and the analysis of the results can be speeded up by using powerful computer programs.

CHAPTER 2. THE THEORY OF COMPLEX FORMATION

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CHAPTER 2. THE THEORY OF COMPLEX FORMATION

In common with most branches of chemistry the study of complex formation in solution has a series of milestones, represented by the names of scientists who contributed to the field.

Werner was the founder of modern co-ordination chemistry, and his paper on the stereochemistry of complexes[80] inspired Bödlander[81] and N. Bjerrum[82], early workers in solution chemistry studying copper(II)-halide and chromium(III)-thiocyanate complexes respectively. The next big advances were the acceptance of the constant ionic medium method and the work of J. Bjerrum[83] and Schwarzenbach[84] on the stepwise formation of complexes, which was followed by the work of Leden[85] on cadmium(II)-halide complexes, the theoretical work of the Rossottis[86] and the important contribution by Sillén, both in the measurement of metal ion hydrolysis constants and in the production of computer programs.

Definition of formation constant.

Consider the formation of a complex $A_pB_qH_r$, where A denotes the ligand, B the metal ion, H protons and p, q and r are indices denoting the number of ligands, metal ions and protons respectively in the complex. In aqueous solution the complex will be associated with a number of water molecules and the formation of the complex can be represented by the following equation :

$$pA(H_2O)_w + qB(H_2O)_x + rH(H_2O)_y \rightleftharpoons A_pB_qH_r(H_2O)_z + (pw + qx + ry - z)\ H_2O \qquad (1)$$

In practice the activity of free water is assumed constant, and equal to unity, and the water of hydration is omitted from equations.

Hence $pA + qB + rH \rightleftharpoons A_pB_qH_r$ (2)

The values of p, q and r are integers, positive or zero for p and q, but r may be negative when hydrolysed species are formed. When the activities of the species present are considered, the equilibria present are definable in terms of a thermodynamic formation constant :

$$\beta^{\circ}_{pqr} = \frac{(A_pB_qH_r)}{(A)^p(B)^q(H)^r} \qquad (3)$$

$$\beta^{\circ}_{pqr} = \frac{[A_pB_qH_r]}{[A]^p.[B]^q.[H]^r} \cdot \frac{f A_pB_qH_r}{fA^p.\ fB^q.\ fH^r} \qquad (4)$$

f is the activity coefficient, curved brackets refer to activities and square brackets to concentrations.

If the activities of all species are held constant, an alternative, but related, formation constant can be defined in terms of the molar concentrations :

$$\beta_{pqr} = \frac{[A_pB_qH_r]}{[A]^p.[B]^q.[H]^r} \qquad (5)$$

A third constant occasionally encountered in the literature is the mixed constant, so called because some terms are concentrations and other terms (usually only the hydrogen ion) are activities.

These simple definitions are not simple to apply in practice and

many problems arise. The use of equation (4) is limited to systems (a) where the results can be extrapolated to zero ionic strength, or (b) where the activity coefficients can be calculated. When there are numerous stepwise species (b) becomes difficult.

Equation (5) is used frequently but the thermodynamics of the system can only be compared to other systems with a similar ionic background. Activity coefficients may be held constant by use of a solution of sufficiently high ionic background salt such that ion pair effects are constant. This can be achieved by using a salt which contains ions having no tendencies to complex with the species being investigated. The usual choice is sodium perchlorate, lithium perchlorate or potassium nitrate.

In monitoring the reaction by potentiometry use is made of the Nernst equation[88]:-

$$E = E^{o} + \frac{RT}{\mathcal{Z}F} \ln\left\{\frac{(A)^{p}(B)^{q}(H)^{r}}{(A_{p}B_{q}H_{r})}\right\} \qquad (6)$$

for the reaction $pA + qB + rH \rightleftharpoons A_{p}B_{q}H_{r}$.

This calls for a definition of unit activity (i.e. the standard state) for the species present. For the glass electrode :-

$$E = E^{o} + \frac{RT}{\mathcal{Z}F} \ln(H) \qquad (7)$$

The choice of a standard state is usually arbitrary and related to the selection of a standard solvent (usually water).

If there is a cell

$$- \; Pt(H_2) \; \left| \begin{matrix} X^-, \; ClO_4^- \\ Na^+, \; H^+ \end{matrix} \right| \; Ag, AgX \; +$$

and the standard solvent is water, then

$$E = E^o_{aq} - \frac{RT}{F} \ln [H^+][X^-] - \frac{2RT}{F} \ln f$$

where $\ln f = \frac{A z^+ z^- \sqrt{I}}{1 + \sqrt{I}} - bI$, z^+ and z^- being the charges on cation and anion, and A and b being constants (Davies equation[88]).

Then $$E^o_{aq} = \text{limit } (E + \frac{RT}{F} \ln H^+ X^-) \quad \text{as } [H^+][X^-] C_{TOT} \to O \qquad (8)$$

This implies :-

$$\text{limit } f = 1 \quad \text{as } [H^+][X^-] C_{TOT} \to O$$

where $C_{TOT} = \frac{1}{2} \sum C_i Z_i = [X^-] + [ClO_4^-] = [H^+] + [Na^+]$

and where f is the mean activity coefficient, as defined above.

If the standard state is chosen as a solvent with defined compostion, then

$$E = E^o_{NaX} - \frac{RT}{F} \ln [H^+][X^-] - \frac{2RT}{F} \ln f^1$$

$$E^o_{NaX} = \text{limit } (E + \frac{RT}{F} \ln [H^+][X^-])$$

$$\text{as } [H^+][ClO_4^-] \to O$$

$$\text{and } [Na^+][X^-] \to C_{TOT}$$

This implies :-

$$\text{limit } f^1 = 1 \quad \text{, where } f^1 \text{ is the mean activity coefficient.}$$
$$\text{as } [H^+][ClO_4^-] \rightarrow 0$$
$$\text{and } [Na^+][X^-] \rightarrow C_{TOT}$$

Hence the obtained results may be extrapolated to the standard solvent, but Biedermann[89] has shown this to be unnecessary, as long as, in 3M $NaClO_4$, the sum of the concentrations of the ions in the reaction $A^- + B^+ \rightleftharpoons AB$ does not exceed 150mM then the error in a potentiometric reading does not exceed $\pm$ 0.1mV. This latter approach is used in the present work.

The Gibbs free energy of the reaction is directly obtainable from the stability constant, according to the reaction isotherm[88]:-

$$\Delta G^o = - RT \ln \beta_{pqr} \qquad (10)$$

where ΔG^o is the Gibbs standard free energy with 3.00M sodium perchlorate as the standard solvent. Further thermodynamic parameters can be obtained by determining β_{pqr} at different temperatures and then using the van't Hoff reaction isochore :-

$$\left(\frac{\partial \ln \beta}{\partial T}\right)_p = \frac{\Delta H^o}{RT^2} \qquad (11)$$

$$\text{Integrating:- } \ln \frac{\beta_1}{\beta_2} = \frac{\Delta H^o}{R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right) \qquad (12)$$

Where subscript 1 refers to the lower temperature and subscript 2

refers to the higher temperature.

The use of the above equation assumes that ΔH^{o} does not vary with temperature, but Izatt et al have observed that this is often not the case[90], so the method is unreliable. Despite this, the method is still used occasionally where no calorimeter is available to measure ΔH^{o} directly.

The enthalpy of formation can be obtained directly by calorimetry, and then by using the Gibbs-Helmholtz equation[88]:-

$$\Delta G^{o} = \Delta H^{o} - T\Delta S^{o} \qquad (13)$$

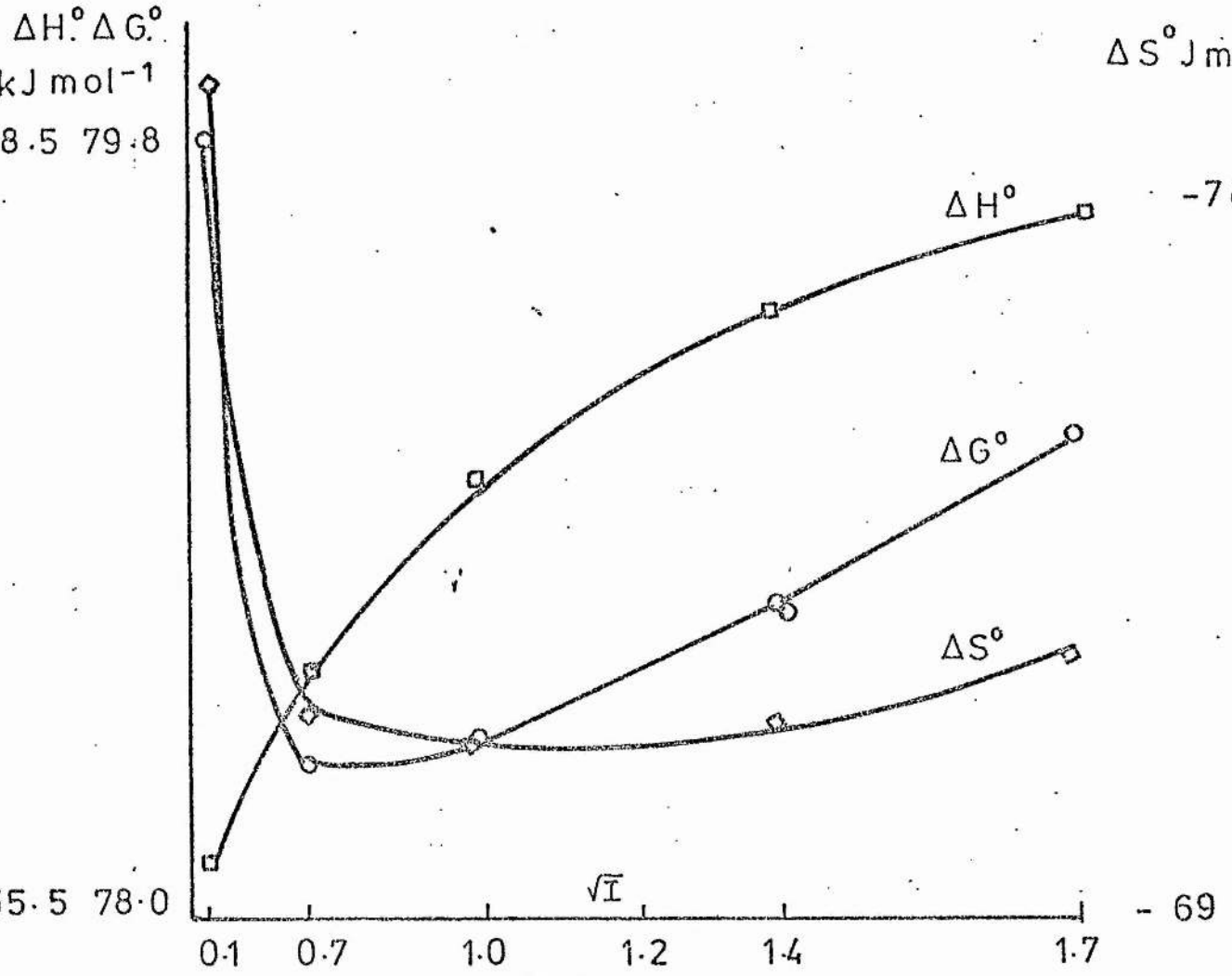
the values for the entropy of reaction may be calculated. The enthalpy change is related to the difference between the bond and solvation energies of the products and bond and solvation energies of the reactants, whereas the entropy change is related to the change in randomness which accompanies the reaction.

Influence of the medium on thermodynamic quantities.

The relationship between the quantities obtained in 3.00M $NaClO_4$ and the quantities ΔG^{o}, ΔH^{o} and ΔS^{o} referring to the more usual standard state, pure water, is worthy of consideration to compare results for the same reaction in different media. The thermodynamics of cadmium(II)-halide and -acetate complexes have been studied at various ionic strengths I[91] and it was found that the thermodynamic quantities are only slightly affected by changes of I in the range 0.30M to 3.00M. The heat of ionization of water, ΔH^{o}_{w}, has also been measured at various ionic strengths[92-93], and this quantity varies linearly with I in the range

0.50M to 3.00M, ΔG^{o}, however, passes through a aminimum at I ~ 0.50M which is due to a sharp increase in the ΔS^{o} term up to 0.50M. This minimum has been noted by Dyrssen et al for several other systems[94].

Figure 1, Variation of ΔG^{o}_{w}, ΔH^{o}_{w} and ΔS^{o}_{w} with $I^{\frac{1}{2}}$



Before going on to consider the significance of the thermodynamic quantities, it might be appropriate to compare advantages and disadvantages of various ionic strengths and working temperatures.

Factors affecting choice of temperature and ionic background.

Examination of the literature reveals a wide range of ionic strengths and working temperatures[95-96]. However, a vast quantity of data has been assembled at a temperature of 25° and in an ionic background

of 0.10 or 0.15M, usually potassium nitrate. Other workers, such as the Sillén school, prefer to use much higher concentrations of ionic background, such as 3.00M sodium perchlorate. The Perrin school, on the other hand works at 37° and 0.15M KNO_3 [97], and uses an activity coefficient of 0.78 instead of 1.00. The present work follows the Sillén school, and previous work in this laboratory, in working at 25° in a background 3.00M in (sodium) perchlorate. The disadvantages of 25° and 3.00M, and 37° and 0.15M are summarised below[28].

25° and 3.00M is far removed from biological blood plasma conditions and any trace impurities in the sodium perchlorate are emphasised at 3.00M, where some ion-pairing effects may still occur. Also, working at 25° increases the ligand pKs, and while those of amino-acids can still be measured, those of other ligands may lie outside the range of glass electrodes.

Although 37° and 0.15M corresponds very closely to blood plasma conditions, one immediate drawback of working at 37° is that volumetric glassware is calibrated for use at 20° (or 25°) and recalibration must be carried out if the work is going to take place at higher temperatures.

The titration assembly needs to be thermostatted at 37°, otherwise condensation appears on the cooler parts of the system and an electrothermal effect is set up in the electrodes which may result in errors of a few millivolts in e.m.f. readings. The tubing linking the burette to the

titration vessel must also be at 37^{o} to minimise temperature fluctuations. Ion-responsive electrodes are less stable at higher temperatures and the rate of hydrolysis of peptides is increased. The activity coefficient is altered if the ion concentration changes by more than 8mM in the background of 0.15M. Finally, the Sillén school reports many metal ion hydrolysis constants at 25^{o} in 3.00M sodium perchlorate[98], but very few have been studied at 37^{o} and 0.15M so preliminary ion hydrolysis studies may be necessary before metal-ligand interactions can be examined.

The significance of the thermodynamic quantities.

a) Enthalpy of formation.

Complex formation is favoured by negative heat changes. Enthalpy changes, which are independent of the composition as a whole[99], in aqueous solution are the heat changes which accompany the replacement of water by other ligands. Heat changes of the order of $-40 kJ\ mol^{-1}$ are attributed to the formation of, essentially, covalent bonds. The change in standard enthalpy, ΔH^{o}, can be considered to consist of two parts, the internal part ΔH_I and the environmental part ΔH_E. The first of these can be considered to be the heat change for the following reaction:-

$$pA_{(g)} + qB_{(g)} + rH_{(g)} \rightleftharpoons A_pB_qH_{r(g)} \qquad (14)$$

To evaluate ΔH_I it is necessary to determine the heats of hydration of the ligand, the metal ion, the proton and the complex, as shown overleaf.

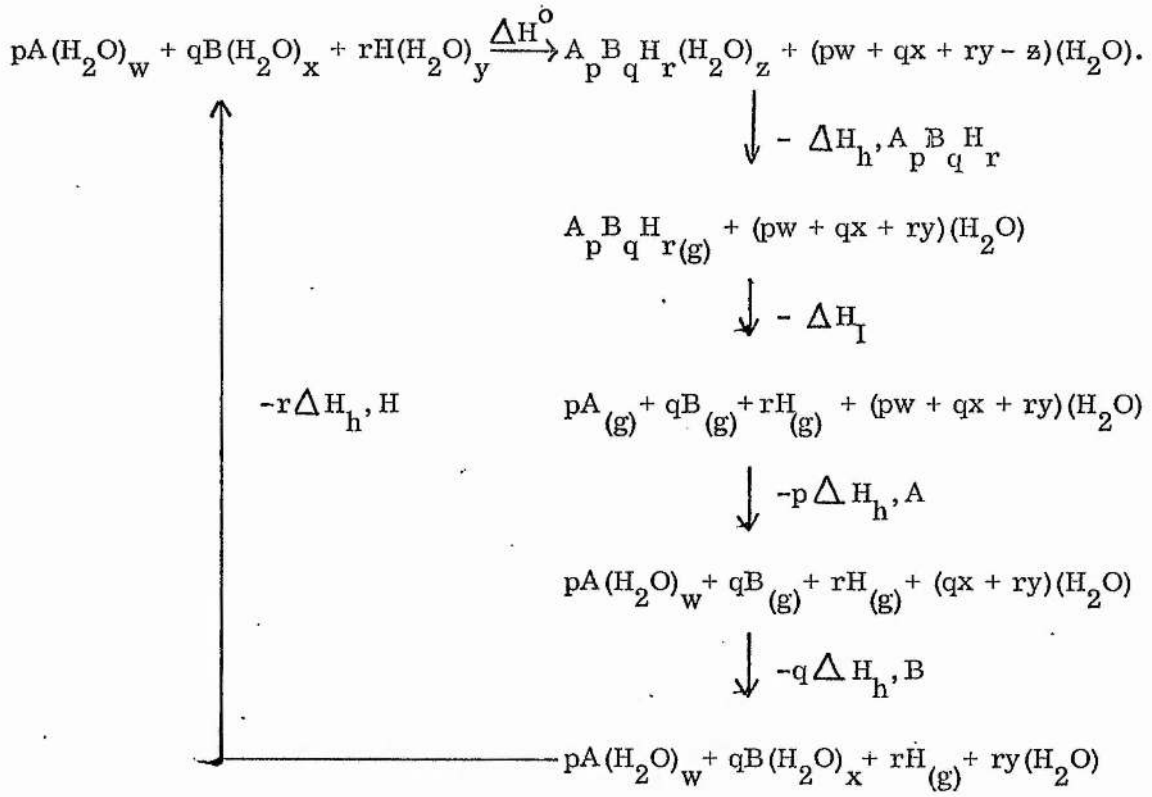


Figure 2. Determination of internal part of ΔH^{o}.

ΔH_h, $A_pB_qH_r$, ΔH_h, B, ΔH_h, A and ΔH_h, H are the heats of hydration of $A_pB_qH_r$, B, A and H respectively.

ΔH_I is then given by Hess' Law.

$$\Delta H_I = \Delta H^{o} + p\Delta H_h, A + q\Delta H_h, B + r\Delta H_h, H - \Delta H_h, A_pB_qH_r \qquad (15)$$

and the environmental part is given by

$$\Delta H_E = \Delta H^{o} - \Delta H_I \qquad (16)$$

The heats of hydration for several metal ions have been reported[100], but values for heats of hydration for ligands and complexes are much more difficult to determine. Values for ligands can be determined from thermodynamic cycles, which involve dissolving the ligand in acid solution.

Unfortunately an experimental value of the electron affinity of the ligand is required[101], and few values exist. At the present time there is no satisfactory method for evaluation of complex ion heats of hydration: however values can be calculated using the Born charging equation[101]:-

$$\Delta H_h = -\frac{Z^2 e^2}{2R}\left(1 - \frac{1}{D} + \frac{T}{D^2}\left(\frac{\partial D}{\partial T}\right)_p\right) \qquad (17)$$

Where Ze = product of charge on ion and electronic charge

R = radius of central ion plus diameter of water molecule (in nm)

D = dielectric constant of medium

T = temperature, K.

The values obtained from equation (17) should be considered only approximate, but can give a guide to trends along a series of metal ions or of similar ligands.

(b) Entropy of formation.

The change in entropy on complexation of the ligand is very dependent upon the ligand. If the ligand has no charge, the solvent will be less ordered around the complex then the metal ion, but if the ligand carries a charge, then on complexing there will be a decrease in the number of ions, neutralisation of the electrical charge, attenuation of the remaining charge and displacement of the water from the hydration sphere of the reactants. For a chelating ligand further factors, such as loss of configurational entropy, must be taken into account. Reactions are favoured

by an increase in entropy, and so the factors favouring complexation are a decrease in the number of ions, neutralisation of the charge and a less ordered solvent. Those factors inhibiting complex formation are the loss of translational entropy to vibrational and rotational entropy and the loss of rotational entropy in polyatomic ligands. For most complex formation reactions, however, the ligational entropy charges are positive[102-103].

c) Gibbs free energy.

The Gibbs free energy is a criterion for the thermodynamic feasibility of a reaction, and ΔG^{o} must be negative if the reaction is to proceed. Its dependence on the enthalpy and entropy is shown in equation (13) and so a reaction is possible with either unfavourable entropy or unfavourable enthalpy as long as the favourable variable predominates.

Table 2. Dependence of ΔG^{o} upon ΔS^{o} and ΔH^{o}[102]

Complex	$-\Delta G^{o}$ (kJ mol^{-1})	ΔH^{o} (kJ mol^{-1})	ΔS^{o} (JK^{-1}mol^{-1})
$(CaOOCH)^{+}$	8.11	4.17	41.8
$(CaOOCCH_3)^{+}$	7.07	3.76	37.6
$(CaSO_4)^{+}$	19.20	19.66	129.6
$(MgOOCH)^{+}$	8.11	-7.50	4.2
$(MgOOCCH_3)^{+}$	7.07	-6.27	4.2
$(CaClO_4)^{+}$	10.87	-49.35	-129.6

It can be seen that a negative ΔG^{o} is not enough to describe a reaction, and ΔH^{o} and ΔS^{o} are valuable parameters to determine.

Methods of calculation.

In the consideration of the formation of the complex $A_p B_q H_r$ (equations (1) and (2)), the Gibbs free energy change is given by equation (10).

$$\text{i.e.} \quad \Delta G^{o} = -2.303RT\log \beta_{pqr}$$

Hence ΔG^{o} is obtained directly from knowledge of the formation constant. In this study the formation constants were obtained by the normal method of following the hydrogen ion concentration during a titration of the reactants A and B. For initial calculations, the function $\bar{Z}$ (sometimes $\bar{n}$), defined as the average number of ligands bound to each central group[86], was plotted against - log a, where a is the free ligand concentration, producing a "formation curve". This treatment of data is only useful for mononuclear complexes, as for polynuclear complexes (including protonated and hydroxy species) the function $\bar{Z}$ has little significance. Mathematically $\bar{Z}$ is defined as:-

$$\bar{Z} = \frac{[AB] + 2[A_2B] + \ldots.}{[B] + [AB] + [A_2B] + \ldots.} = \frac{\sum_{1}^{N} n\beta_n a^n}{\sum_{0}^{N} \beta_n a^n} \qquad (18)$$

Practically $\bar{Z}$ can be determined from knowledge of the total concentrations A, B and H in solution, the values of the formation constant for any species AH_r and the free hydrogen ion concentration h.

From (18), $\bar{Z} = \frac{\text{Bound ligand concentration}}{\text{Total metal concentration}}$

Bound ligand concentration = Total ligand - (free ligand + protonated ligand)

$$= A - (a + \sum_{1}^{N} a\beta_n h^n)$$

$$= A - a(1 + \sum_{1}^{N} \beta_n h^n) \qquad (19)$$

The free ligand concentration, a, is derived from a mass-balance equation:-

$$H = h - \frac{wk}{h} + \sum_{1}^{N} n[AH_n]$$

$$= h - \frac{wk}{h} + \sum_{1}^{N} an\beta_n h^n$$

$$\text{i.e. } a = \frac{H - h + \frac{wk}{h}}{\sum_{1}^{N} n\beta_n h^n} \qquad (20)$$

Hence $\bar{Z}$ can be obtained from (19) and (20).

The transformation of plots of $\bar{Z}$ against - log a into formation constants by graphical means has been extensively reviewed by Rossotti and Rossotti[86]. The methods used in this study were numerical, employing high speed digital computers. The aim was to reproduce the experimental $\bar{Z}$, - log a curve using constants obtained from the computer programs.

The change in enthalpy, ΔH^o, was measured directly by calorimetry. The concentrations of the species in the calorimeter were determined by using the computer program HALTAFALL[104], and changes in concentrations together with the necessary corrections for heats of

formation of water, of deprotonation and hydrolysis, were calculated using RWCALCRD (see next chapter).

This treatment yields experimental parameters, heat evolved and change in concentration of species under study, from which the change in enthalpy can be calculated numerically or graphically. A plot of heat evolved per mole of metal ion against the degree of formation, $\bar{Z}$, will yield $\triangle H_1^o$ at $\bar{Z} = 1$, $\triangle H_2^o$ at $\bar{Z} = 2$ etc., for simple systems with well separated heats. $\triangle S^o$ can now be calculated from equation (13).

CHAPTER 3. COMPUTATIONAL ASPECTS

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CHAPTER 3. COMPUTATIONAL ASPECTS

The computer and chemistry.

With the advent of the computer, the chemist's approach to numerical problems has been revolutionised. Crystallography was the first branch of chemistry to benefit from the use of the computer more than 20 years ago[105], as the type of calculation involved, though reasonably straightforward, was both long and tedious. Perhaps the biggest strides taken in chemistry, using a computer, have been in the field of quantum mechanics. The large storage space and the speed at which calculations can be performed in the latest generation of computers have enabled quantum chemists to tackle hitherto impossible calculations.

Computers are extensively used in analytical chemistry[106] for processing data obtained by physical methods. Libraries of information can be set up for chromatography, mass spectrometry, nmr and chemical literature. Programs exist for phase equilibria[104], nmr[107], esr[108] and calorimetry[109], where trial parameters are used to produce simulated distribution curves, spectra and thermograms respectively. General curve analysis is also employed in spectroscopy and electroanalytical chemistry.

Another area of chemistry to feel the impact of the computer is the calculation of formation constants by various numerical methods. These computations have been the subject of a number of reviews[110-111], and have aroused a great deal of interest in solution chemistry in recent years.

The calculation of formation constants by computer.

Many programs, of varying complexity, have been written to determine formation constants, and the type of program chosen depends on several factors. For simple systems, A_nB or AH_m, non-statistical programs will suffice[112], although if the constants overlap a linear least-squares technique is more applicable. For more complicated systems, where polynuclear, hydrolysed or protonated species are likely to be present, a more general non-linear least-squares program must be used. The availability of a high-speed digital computer with a large storage capacity is a criterion for the use of the latter type of program. As the present work included a search for protonated and hydrolysed binary species, and ternary species, a general program was required to calculate the stability constants. Two such programs, SCOGS[113], and LETAGROP VRID[114-116] have been described in the literature, and a third program, MINIQUAD[117], has recently been made available. A FORTRAN version of LETAGROP, LGVRID, was stored on the St. Andrews IBM 360/44 computer along with versions of SCOGS and MINIQUAD.

Most of the present work used SCOGS, because its mechanics were less complex than those of the other programs, but comparisons between the results obtained from the different programs have been made.

SCOGS, LETAGROP and MINIQUAD all depend upon Taylor's series, a mathematical method of expressing a function in terms of its derivatives.

If $y = f(k_r; a_r)$ where $r = 1$ to M, (1)

where y is a measured quantity,

a_r = variable, but accurately known, quantities,

k_r = unknown constants,

then Taylor's series gives the following expression:-

$$U = U_o + \sum_1^M \left(\frac{\partial U}{\partial k_r}\right) \delta k_r + \sum_1^M \frac{\left[\partial\left\{\sum_1^M \left(\frac{\partial U}{\partial k_r}\right) \delta k_r\right\}\right]}{\partial k_r} \qquad (2)$$

where U is the error square sum:-

$$U = \sum_1^M \left\{y - f\left(\sum_1^M k_r ; \sum_1^M a_r\right)\right\}^2$$

The "best" values are those which minimise this sum, i.e. U_o.

At this point the prgrams begin to differ in rigour.

LETAGROP VRID.

In this program equation (2) is the equation of a "pit" with minimum point U_o. The values of k_r are incremented to give not only the minimum point, but also a map of the surroundings. The function y is $\bar{Z}$ and so the quantity minimised is:-

$$U = \sum_1^M \left(\bar{Z}_{calc} - \bar{Z}_{expt}\right)^2 \qquad (3.L).$$

Equation (2) represents a generalised ellipsoid in (M + 1) dimensional space. A measure of the spread of data around U_o is enclosed in the "D boundary"[118]:-

$$U = U_o + \frac{U_o}{n - M} \tag{4}$$

where n is the number of experimental points, and as $n \gg M$ in general:-

$$U = U_o + \frac{U_o}{n} \tag{5}$$

The D boundary is again a generalised ellipsoid, but this time in M dimensional space with the least squares point, k_r, at its centre. The "standard deviation" can be calculated from the D boundary because it is identical with the range of values each k_r can assume on the boundary. The VRID block was added to overcome the problem of a "skew pit" which arises from correlation of shifts and causes incorrect convergence. The shifts are altered by a "twist matrix" so that they are made along the axes of the skew pit rather than the co-ordinate axes.

SCOGS.

This is a more general program than LETAGROP VRID when applied to stability constant work, because it can deal with up to twenty complexes of the type $A_p A'_{p'} B_q B'_{q'} H_r$. For the jth complex this leads to a stability constant:-

$$\beta_j = \frac{\left[A_{p_j} . A'_{p'_j} . B_{q_j} . B'_{q'_j} . H_{r_j}\right]}{[A]^{p_j}[A']^{p'_j}[B]^{q_j}[B']^{q'_j}[H]^{r_j}} \tag{6}$$

or $$C_j = \beta_j . a^{p_j} . a'^{p'_j} . b^{q_j} . b'^{q'_j} . h^{r_j} \tag{7}$$

There is provision for use of the activity coefficient of the hydrogen ion[113], hence SCOGS can give mixed constants.

For N complexes

$$A = \sum_{1}^{N} (a + p_j \beta_j C_j) \tag{8}$$

$$A^1 = \sum_{1}^{N} (a^1 + P^1_j \beta_j C_j) \tag{9}$$

$$B = \sum_{1}^{N} (b + q_j \beta_j C_j) \tag{10}$$

$$B^1 = \sum_{1}^{N} (b^1 + q^1_j \beta_j C_j) \tag{11}$$

If a, a^1, b and b^1 were known, A, A^1, B and B^1 would be the experimental values, but as they are not known let

$f(a) = A_{expt} - A$ (equal to zero for true values of a, a^1, b and b^1)

$$f(a) = A_{expt} - (a + \sum_{1}^{N} p_j \beta_j C_j) \tag{12}$$

and similarly,

$$f(a^1) = A^1_{expt} - (a^1 + \sum_{1}^{N} p^1_j \beta_j C_j) \tag{13}$$

$$f(b) = B_{expt} - (b + \sum_{1}^{N} q_j \beta_j C_j) \tag{14}$$

$$f(b^1) = B^1_{expt} - (b^1 + \sum_{1}^{N} q^1_j \beta_j C_j) \tag{15}$$

Values of a, a^1, b and b^1 (and hence C_j, equation (7)) are now

obtained by a Newton-Raphson iterative procedure. This minimises functions (12-15) by calculating shifts that will simultaneously minimise all four functions. Progressing through each point, least squares equations are built up and solved by matrix inversion, to yield shifts in constants. These shifts are obtained from Taylor's series, but unlike the pit-mapping procedure only first-order terms are used. The quantity minimised is:-

$$U = \sum_{1}^{M} (\text{Titre}_{\text{expt}} - \text{Titre}_{\text{calc}})^2 \qquad (3.S)$$

The calculated titre is obtained from the current set of constants and the experimental value of the hydrogen ion concentration.

The original SCOGS has been amended somewhat[119-120], mainly in input and output. The input now deals with any titrant and the output consists of several calculated functions such as $\bar{Z}$. A variant of SCOGS, GSCOGS, accommodates up to three ion-sensitive electrodes[121].

MINIQUAD.

This is the latest program to be developed for calculating formation constants. Compared with SCOGS the program varies sets of β (rather than $\log\beta$), minimises the sums of the squares in analytical concentrations rather than titre volumes, can handle several, rather than three, electrodes simultaneously, and approaches the least squares minima using the Fletcher and Powell steepest descent method rather than a Jacobean matrix.

Let there be nk parameters (formation constants) β_j of which n are to be determined. For each of x reactants there is a mass-balance

equation:-

$$T_i = C_i \sum_i a_{i_j} C_j \qquad (16)$$

where T_i is the total concentration of reactant i, C_i is the concentration of free (uncomplexed) reactant, and C_j is the concentration of the complex j as shown by:-

$$C_j = \beta_j T_k C_k^{q_{k_j}}$$

The indices q_{k_j} are the stoicheiometric coefficients of the reactions. There are x mass-balance equations at each point, and the sum of squares, U, to be minimised is given as:-

$$U = \sum_1^M (T_i^{calc} - T_i^{obs})^2 = \sum_1^M (\Delta T_i)^2 \qquad (3.M)$$

Thus U is the sum of squared residuals for all the mass-balance equations. These residuals are analysed upon convergence to see how nearly they follow a normal distribution. The final output also includes a complete species distribution analysis.

Being a new program, MINIQUAD has been less extensively tested with experimental data than either SCOGS or LETAGROP.

SCOGS or LETAGROP?

Although both the Gaussian and "pit mapping" approaches have been in use for several years, no conclusions have been reached as to which method is "best". Naturally the Sillén school favours "pit-mapping" and Perrin favours Gaussian, but Tobias[122], who could be assumed to be

unbiased, as he has used both methods, prefers Gaussian. Further criteria are mathematical rigour, ability to define difficult systems and avoidance of false minima, but experimental parameters must ultimately be reproduced from the obtained constants. LETAGROP was devised as a "supplement to graphical methods"[114], but since 1962 it has become a method in its own right. SCOGS was conceived as a numerical method, and if used correctly should be the faster method. However the applications of LETAGROP extend far beyond potentiometry.

Comparison between SCOGS and MINIQUAD.

A comparison between SCOGS and LETAGROP has been made previously[119]; as MINIQUAD was used in this work in place of LETAGROP, a comparison between constants produced from data in SCOGS and MINIQUAD was made.

a) Protonation of threoninate.

The data were obtained from the potentiometric investigation of the protonation of the threoninate anion ; the collection of the data is discussed in Chapter 7. The experimental readings of titre and e.m.f. were used in the two programs to determine constants for the following equilibria:-

$$H^+ + A^- \rightleftharpoons HA \qquad K_{a_1} = \frac{[HA]}{[H^+][A^-]}$$

$$H^+ + HA \rightleftharpoons H_2A^+ \qquad K_{a_2} = \frac{[H_2A^+]}{[H^+][HA]}$$

The results are given in Table 3.

Table 3. Protonation of threoninate

	SCOGS	MINIQUAD
pK_{a_1}	9.348 ± 0.006	9.359 ± 0.006
pK_{a_2}	2.577 ± 0.004	2.579 ± 0.004

The two programs therefore give consistent results for this data.

(b) Dimerisation of acetate.

The data were obtained from the potentiometric investigation of the dimerisation of the acetate anion[123]. The following are the equilibria involved and the published results.

$$H + A \rightleftharpoons HA \;,\; \log \beta_{11} = 6.965$$

$$H + 2A \rightleftharpoons HA_2 \;,\; \log \beta_{12} = 7.046$$

$$2H + 2A \rightleftharpoons H_2A_2 \;,\; \log \beta_{22} = 13.396$$

These results were obtained by both graphical methods and LETAGROP. They were also given by MINIQUAD, but no convergence could be obtained in SCOGS despite using a variety of values for the input constants. Convergence was obtained when only two constants were used, but the standard deviation was so large that the results were meaningless.

This lack of convergence was examined more closely by using a simulated set of data obtained from HALTAFALL. This data was designed so that a metal-ligand interaction was examined, as in SCOGS' original

conception, and two constants were arranged to lie close together.

(c) Simulated Data.

The hypothetical system consisted of five equilibria, including the threoninate equilibria shown in (a). The other three equilibria were

$$A + B \rightleftharpoons AB \qquad \log \beta_1 = 6.965$$

$$2A + B \rightleftharpoons A_2B \qquad \log \beta_2 = 7.046$$

$$3A + B \rightleftharpoons A_3B \qquad \log \beta_3 = 13.396$$

Nine experiments were simulated giving a total of 99 readings. The initial metal and ligand concentrations were as shown in Table 4.

Table 4 Simulated data for testing SCOGS

Initial metal concentration (mM)	Initial ligand concentration (mM)
20.0	40.0
20.0	20.0
20.0	10.0
10.0	30.0
10.0	20.0
10.0	10.0
5.0	25.0
5.0	15.0
5.0	5.0

For all simulated titrations the initial mineral acid concentration was equal to the metal concentration, the initial volume was 25.0 ml,

the titrant was sodium hydroxide (40.0 mM), and each addition was of 2.0 ml stepwise up to a total of 20.0 ml.

Convergence was unobtainable using SCOGS demonstrating that the program is suspect when formation constants lie close together. Therefore, when dealing with such systems, such as D - and L - amino-acids, SCOGS should not be used.

Other Computer Programs.

During the course of the present work, use was made of other computer programs and a brief description of these is given below.

HALTAFALL.

This program has previously been used for predicting concentrations of species from formation constants in this laboratory[124]. Recently the existing program has been modified, and a plotter routine added to enable graphs of simulated $\bar{Z}$ versus simulated -log a to be drawn[125]. Comparisons between experimental data and theoretical data obtained by using the experimentally determined constants can now be made.

RWCALCRD.

This was originally RWCALCOR,[119] which had three versions, compressed into one version with three options and with the input tidied up. From an input consisting of the concentrations of the complexes under study, obtained either from SCOGS or HALTAFALL, and the heat of formation of water, this program calculates the change in concentration of each species and heat corrections for formation of water, protonation and

hydrolysis. Up to 20 complexes of type $A_pB_qH_r$, where p, q, and r are positive or zero, but r can also be negative, can be handled. The program can be used to determine heats of protonation, heats of hydrolysis and heats of formation of $A_pB_qH_r$ simultaneously, but better use of the program involves obtaining values of the heat of formation of AH_r and B_qH_r (where r is negative) from separate experiments and these values should then be used to calculate corrections for experiments to obtain the heats of formation of the more complex $A_pB_qH_r$ species. The output, pertinent to the calculation of heats of formation, is the corrected experimental heat, Q, associated with the formation of x complexes, and the changes in concentrations, n_x of these complexes. For the N th point:-

$$Q_N = \sum_1^X (\Delta H_x . \Delta n_x)_N$$

where Q_N is measured in joules, ΔH_x is in J mol^{-1} and Δn_x is in mol.

For x complexes, x values of N are needed to solve for the heats of formation but more values than this are determined experimentally, and then the following "least-squares" method can be used.

Suppose that the "best" heats are ΔH_1^1, ΔH_2^1, ΔH_3^1 etc.

i.e. $$\sum_1^x (\Delta H_x^1 . \Delta n_x)_N - Q_N = 0$$

Then let $$F = \sum_1^M \left\{ Q_N - \sum_1^x (\Delta H_x^1 . \Delta n_x)_N \right\}^2$$

The "best" values of the heats are obtained by minimising the function:-

$$\frac{\partial F}{\partial (\Delta H_x^1)} = O = - \sum_1^M \left\{ 2 \Delta_{n_x} (Q_N - \sum_1^X \Delta H_x^1 . \Delta_{n_x}) \right\}$$

There will be a function of this type which can be solved as simultaneous equations to give values of ΔH_x^1 which are the "best" constants for the set of experimental results 1 to M.

A program RWSOLV[119] was available to calculate ΔH_x^1 in this manner and was added on to the end of RWCALCRD to facilitate speedy calculation.

RWZPLOT.

This program was originally called RWZASCOG and calculated the experimental $\bar{Z}$ and - log a for every point, using the same input data as SCOGS. A plotter routine was added to it in 1972, and a simple version of the program, without a plotter routine, is in use in the undergraduate teaching laboratories in this department[126].

RWCOMPLT.

This program, the version of COMICS[127] mounted on the computer in St. Andrews, calculates equilibrium concentrations of all species in multi-ligand-multi-metal systems, using as input data the pH of the solution, the total concentrations of each metal ion and ligand and the logarithm of the formation constant for each complex. Mixed ligand, hydrolysed, protonated and polynuclear species can be handled and the program limits are ten metal ions, ten ligands, 100 formation constants and 50 pH values. The input has been modified from the published version

as has the output which now has three plotter routines available.

Interpretation of data using the computer.

Although considerable advantages can be ascribed to the use of a computer, pitfalls exist. A degree of knowledge of computer languages is necessary to avoid simple time-consuming errors such as format errors. There should be good access to the computer, in order to be able to take advantage of the peripheral facilities and so evade unnecessary duplication of card punching etc. The use of trial parameter programs such as SCOGS and LETAGROP is a technique in its own right and must be mastered. The usual criterion in this type of program is that the set of constants giving the smallest standard deviation is the "best" set of constants to describe the experimental data, but this must not be imposed too rigorously as it is possible to obtain a constant which describes a chemically nonsensical species, or a constant which gives concentrations of a species which has no physical significance. A knowledge of the program combined with a degree of chemical intuition and knowledge can give meaningful results, although the recognition of poor results is equally as important as the interpretation of good results. Editing of results should be confined to pruning results from areas of low experimental accuracy, e.g. e.m.f. readings below pH2 where a glass electrode becomes unreliable.

To chemists the computer has become as important a tool as some spectroscopic techniques. The speed of calculation and the use of iterative procedures are useful and relevant to chemical calculations, and the

presence of a computer, though not absolutely necessary, certainly helps.

CHAPTER 4. EXPERIMENTAL TECHNIQUES

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CHAPTER 4. EXPERIMENTAL TECHNIQUES

Potentiometry and calorimetry were carried out in special vessels (see Chapters 5 and 6) using solutions prepared and analysed by the methods outlined below.

Water.

All the water used was de-ionised by passage through an "Elgastat" (a sulphonated polystyrene ion-exchange resin), then boiled and cooled under an atmosphere of oxygen-free nitrogen. The resistivity of the water was then better than $2M\Omega cm^{-1}$.

Sodium perchlorate.

This substance, the inert background salt, contained heavy metal ions which form complexes with the ligands being used, so leading to errors in stability constant measurement by as much as 1%. Purification of the salt was achieved by the following method:- Solutions of sodium perchlorate were made by either dissolving the monohydrate (Merck "Puriss" or B. D. H. AnalaR) in water, or by neutralising perchloric acid (Fisons A. R.) with sodium carbonate (Fisons A. R.). The solution ($\simeq$ 7.5M) was then made alkaline (pH 9-10) by addition of sodium hydroxide (B. D. H. AnalaR), and allowed to stand for at least seven days. Silica, heavy metal oxides and hydroxides precipitated during this time and were removed by filtration through micropore filters (5000 nm and 450 nm hole diameter-Millipore Ltd.). Carbon dioxide was then removed by making the solution acidic, boiling and then cooling under nitrogen. From this point either crystals of $NaClO_4$ were made or a standard solution was prepared and analysed.

(a) Preparation of crystals:- The solution was adjusted to neutrality with sodium hydroxide (B. D. H. AnalaR) and heated in an evaporating basin to 140°C. After cooling to 105°C the resultant slurry was filtered through a sintered glass funnel (porosity 3) and the crystals of sodium perchlorate were dried in an oven at 105°C.

(b) Preparation of a solution:- After boiling and cooling the solution was made neutral and analysed by cation exchange[128a] and flame photometry[128b].

Perchloric acid.

Concentrated perchloric acid (60-62% w/v, Fisons A. R.) was diluted to make a stock solution of ca 3M. This solution was analysed by titration against sodium carbonate (bromothymol blue as indicator)[128c] and checked with sodium hydroxide solution.

Sodium hydroxide.

1.00M and 0.100M solutions were obtained from ampoules (BDH concentrated volumetric solutions) and their molarities were checked against standard acid solution[128d] and potassium hydrogen phthalate (Fisons A. R.)[128e].

Metal ion solutions.

Metal perchlorates (G. F. Smith, Chemical Co.) were dissolved in perchloric acid to prevent hydrolysis, allowed to stand for several days, filtered through micropores and analysed as follows:-

Zinc(II) :- Quinaldinate[128f] and EDTA (eriochrome black T as indicator)[128g].

Copper(II) :- Electrodeposition[128h] and iodometry[128i].

Nickel(II) :- Electrodeposition[128j] and EDTA (murexide as indicator)[128k].

Cobalt(II) :- Electrodeposition[128l] and EDTA (xylenol orange as indicator)[128m].

Manganese(II) :- Ammonium phosphate[128n] and EDTA (eriochrome black T as indicator)[128o].

Iron(II) :- Commercial iron(II) perchlorate was found to contain up to 4% iron(III), hence iron(II) perchlorate was prepared by dissolving iron sponge (Johnson-Matthey Chemicals "Specpure") in standard perchloric acid[129]. The solution was analysed by oxidation with potassium dichromate (barium diphenylamine sulphonate as indicator)[128p], and the results obtained agreed with the weight of iron dissolved. The solution was stable in absence of oxygen, but oxidised to Fe(III) within 24 hours upon addition of the ionic background.

The hydrogen ion concentration in these solutions, which was approximately equal to the metal ion concentration, was obtained by means of Gran plots[130].

<u>EDTA.</u>

The disodium salt of ethylenediaminetetra-acetic acid is available as a primary standard, hence solutions of this substance could be prepared by direct weighing[128q].

Ligands.

L-asparagine, L-glutamine, L-threonine (B. D. H. Biochemical Grade) and L-histidine (Koch-Light) were dried and analysed.

(a) L-asparagine. H_2O (m.p. 233-235°C, lit. 235°C)

Found :- C, 31.9; H, 7.0; N, 18.5% $C_4H_{10}N_2O_4$ requires C, 32.0; H, 6.7; N, 18.7%.

(b) L-glutamine. (m.p. 183-185°C; lit. 185-186°C (decomp.))

Found :- C, 14.0; H, 6.8; N, 19.2%. $C_5H_{10}N_2O_3$ requires C, 41.1; H, 6.9; N, 19.2%.

(c) L-threonine (m.p. 250°C; lit. 251-253°C)

Found :- C, 40.3; H, 7.6; N, 11.7%. $C_4H_9NO_3$ requires C, 40.3; H, 7.6; N, 11.8%.

(d) L-histidine (m.p. 285-286°C; lit. 287°C (decomp.))

Found :- C, 46.2; H, 6.0; N, 27.2%. $C_6H_9N_3O_2$ requires C, 46.4; H, 5.8; N, 27.1%.

These ligands were used without further purification.

Nitrogen.

Oxygen-free nitrogen (British Oxygen) was further de-oxygenated by passage through chromium(II)chloride and then "scrubbed" in 3.00M sodium perchlorate, all solutions being thermostatted at 25°C.

Volumetric apparatus.

Volumetric apparatus (E-mil Green Line, M. J. Elliot) was provided with calibration certificates. Several calibrations were checked, but all were found to agree with the certificates. The apparatus was

calibrated at 20°C hence all solutions were thermostatted at this temperature before use.

All apparatus was cleaned regularly with "Quadralene" (Quadralene Chemical Products) and alcoholic potassium hydroxide. Before use apparatus was washed with demineralised water, "Elgastat" water, alcohol and anaesthetic ether, and then dried by suction.

CHAPTER 5. POTENTIOMETRY

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CHAPTER 5. POTENTIOMETRY

Description of apparatus

Potentiometric measurements were carried out at 25.0°C in an ionic backgound of 3.00M (sodium) perchlorate. The hydrogen ion concentration was followed by using a cell of the type

Hg, Hg_2Cl_2 | saturated NaCl ‖ test solution | glass electrode.

A sodium chloride rather than potassium chloride salt bridge was employed because potassium ions form insoluble potassium perchlorate in the porous plug of the calomel electrode.

The two types of electrode used were glass (Activion 17SB) and calomel (Activion RCB) together with a digital pH meter (Radiometer Copenhagen PHM 52), which gave e.m.f. readings reproducible to 0.1mV.

The double-walled potentiometric vessel (Pye-Ingold No 604; inner wall pyrex glass, outer wall plastic) was of about 100ml capacity and thermostatted by water at a temperature of 25.0 ± 0.1°C. The vessel was closed by a plastic cover through which the electrodes, the burette tube and the nitrogen inlet were inserted into the solution. The titrant was added from a 10ml piston burette (Metrohm E274); the outlet of the capillary end of the burette was below the surface of the solution in the vessel. The titrant was added in portions of 0.2ml and the e.m.f. was measured after each addition (up to 50 points per titration). Rapid mixing of the solution was achieved by magnetic stirring, using a PTFE follower.

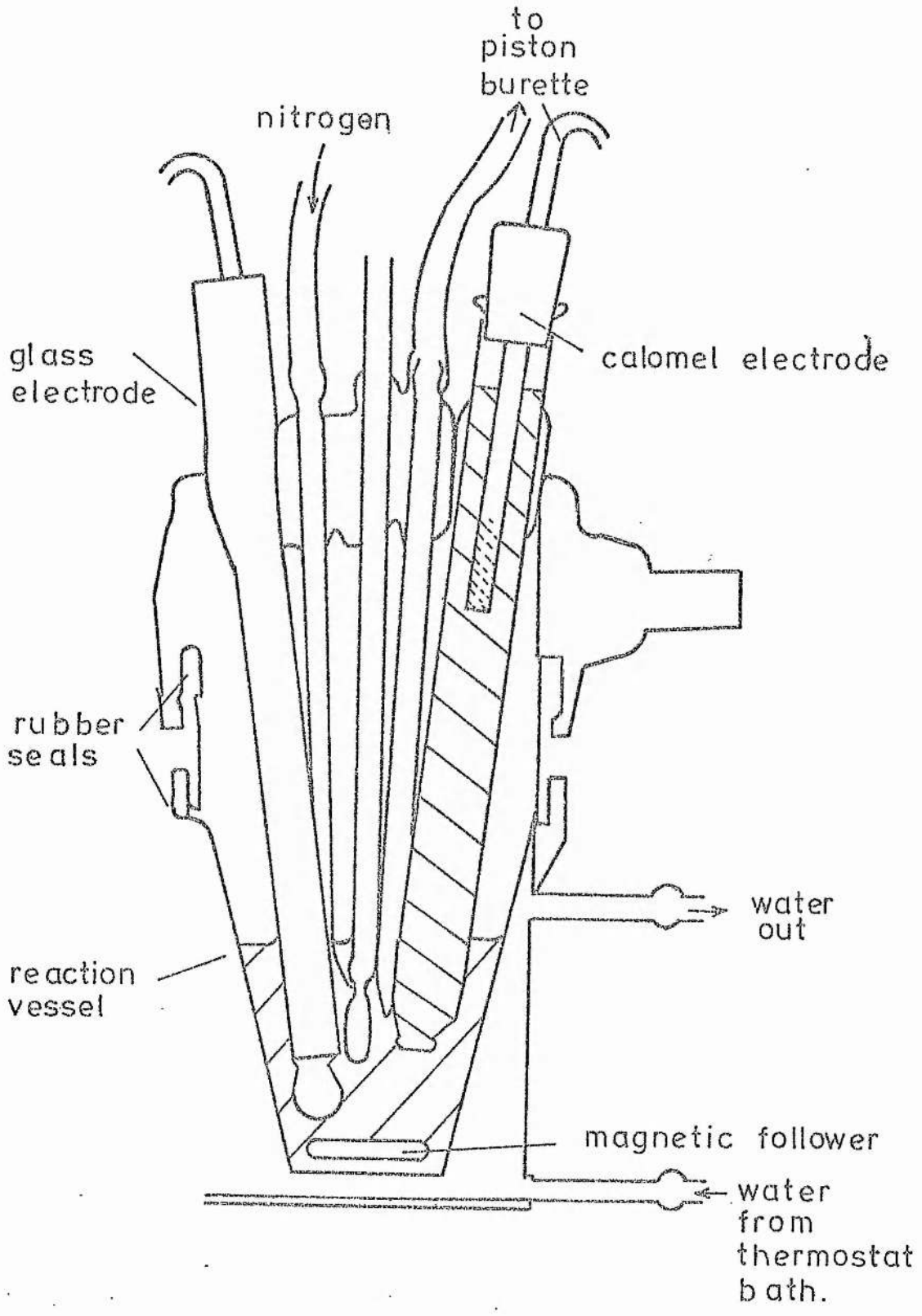


Figure 3. Potentiometric Vessel.

Calibration of the electrode pair.

The electrode pair was calibrated to respond to hydrogen ions by a titrimetric procedure. The e.m.f. of the cell in use is given by:-

$$E = E^{o} + \frac{RT \ln h}{\nu F} + E_j$$

i.e. $$E = E^{o} + 59.162 \log h + E_j \text{ (mV) at } 25^{o}C$$

where E is the e.m.f. as measured on the voltmeter,

E^{o} is a constant,

E_j is the electrode junction potential which arises because of the use of ions of differing mobilities on either side of the liquid junction; i.e. perchlorate, hydrogen or hydroxyl ions in the test solution, and chloride ions in the electrolyte in the calomel electrode. Biedermann and Sillén have measured E_j in 3.00M $(Na)ClO_4$ and have found it to be a function of hydrogen in concentration[131]. For $h < 300mM$, $E_j = -0.016h$ and for $h > 300mM$, $E_j = -0.016h - 0.004(h-300)$.

If E_j is constant, a plot of E versus -log h ought to produce a straight line having slope = $- 59.162mV(-\log h)^{-1}$.

In the calibration titration perchloric acid (24.97ml of concentration 7.250mM) in the vessel was titrated with sodium hydroxide (500.0mM) from an "Agla" syringe, both solutions being 3.00M in $(Na)ClO_4$. Values of $-\log h > 7$ were calculated using a value for $-\log K_w = 14.21$[132]. As can be seen from figure 4 the E versus -log h plot is linear in the ranges -log h = 1.7 - 3.5 and 10.0 - 12.0.

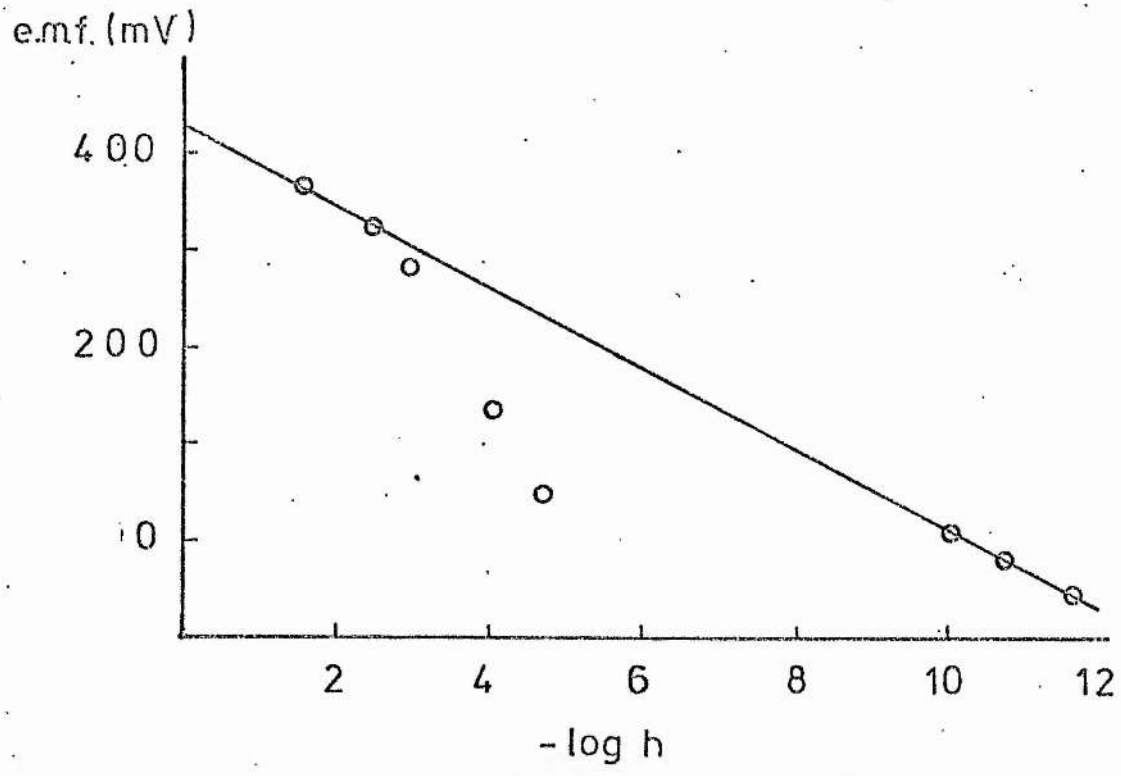


Figure 4 Electrode Calibration Curve.

The deviation from linearity in the intermediate range is due to unbuffered solutions being affected by the borosilicate ions in the glass of the electrodes, and has been observed previously by Williams and Williams[133]. A linear plot parallel to the unbuffered line, in the intermediate range, was obtained using buffers of pH = 4.00, 6.86, 7.42 and 9.18, and therefore E_j was constant over the entire range -log h = 2.0 - 12.0.

A value of E^o for the electrode pair was obtained by extrapolation of the linear portion of the unbuffered curve to meet the E axis. The E^o value was checked using a standard acid solution before and after each subsequent metal ligand titration. This value was found to vary slightly from day to day, the variation being larger when either of the electrodes was very new (two or three weeks) or very old (on average about six months).

Formation constants for the protonation of asparaginate.

These were measured by Williams et al[134] under the same conditions as the present work.

These values were :-

$$\log \beta_{101} = 9.303 \pm 0.018$$

$$\log \beta_{102} = 11.888 \pm 0.022$$

The first β value refers to the protonation of the amino group and the second to the protonation of the carboxylate group.

Formation constants for Zn(II)-asparaginate complexes.

The hydrogen ion concentration in the vessel was varied by titrating alkali into a mixture of metal ion, ligand and mineral acid (titration Nos. 1-13). Two other sets of conditions were also used.

(a) Ligand and alkali titrated into metal ion and mineral acid (titration No. 14)
(b) Metal ion and mineral acid titrated into ligand and alkali (titration No. 15)

Table 5 Experimental results for the Zn(II)-asparaginate system.

Titration number	Initial concentrations (mM) Zn(II)	ligand	mineral acid	Initial volume (ml)	E^o (mV)
1	2.345	9.440	2.218	24.97	411.5
2	2.345	4.777	2.218	24.97	409.5
3	2.345	2.272	2.218	24.97	409.5
4	5.872	23.57	5.552	24.97	416.9
5	5.872	11.75	5.552	24.97	413.0
6	5.872	5.925	5.552	24.97	410.2
7	11.29	44.78	10.67	24.97	410.6
8	11.29	22.26	10.67	24.97	408.9
9	11.29	11.32	10.67	24.97	410.6
10	28.18	114.2	26.63	24.97	412.5
11	28.18	56.94	26.63	24.97	412.5
12	28.18	28.84	26.63	24.97	410.7
13	45.10	45.02	42.62	24.97	411.7

In titrations 1-9 the concentration of the titrant alkali was 39.90mM and in 10-13 200.0mM.

Titration No. 14 - In vessel-[Zn(II)] = 28.21mM; [mineral acid] = 26.61mM
In burette[asn] = 163.8mM; [alkali] = 40.01mM
Initial volume 24.97ml; E^o = 444.4mV

Titration No. 15 - In vessel-[asn] = 166.8mM; [alkali] = 20.00mM
In burette-[Zn(II)] = 28.21mM; [mineral acid] = 26.61mM
Initial volume 24.97ml; E^o = 437.5mV

Titration No. 1

vol. added (ml.)	e.m.f. (mV)
1.29	156.3
1.31	153.5
1.36	143.8
1.38	139.0
1.40	134.5
1.43	127.2
1.45	120.9
1.50	108.5
1.55	98.1
1.60	89.4
1.70	75.7
1.75	70.6
1.80	65.8
1.90	57.9
2.00	50.8

Titration No. 2

1.30	158.8
1.35	143.8
1.40	124.4

Titration No. 2 contd.

vol. added (ml.)	e.m.f. (mV)
1.45	104.8
1.50	88.9
1.55	77.1
1.65	60.7
1.70	54.6
1.75	49.2
1.80	44.4
1.85	39.9
1.90	36.0
1.95	32.1
2.00	28.7

Titration No. 3

1.37	122.9
1.40	105.3
1.43	88.4
1.46	76.6
1.49	66.9
1.55	52.2
1.60	43.3

Titration No. 3 contd.

vol. added (ml.)	e.m.f. (mV)
1.65	35.2
1.70	29.4
1.75	23.4

Titration No. 4

4.70	92.0
4.80	88.4
4.90	85.1
5.00	81.9
5.20	76.3

Titration No. 5

3.30	168.9
3.40	157.9
3.50	144.1
3.55	136.4
3.60	128.4
3.65	121.0
3.70	114.7
3.75	108.9

Titration No. 5 contd.

vol. added (ml.)	e.m.f. (mV)
3.80	103.7
3.85	99.2
3.90	95.2
3.95	91.6
4.10	82.5
4.20	77.3
4.30	72.6
4.50	65.0
4.70	58.1
4.90	52.0
5.00	49.2

Titration No. 6

vol. added (ml.)	e.m.f. (mV)
3.30	180.8
3.40	168.8
3.45	160.7
3.50	151.3
3.55	138.3
3.60	126.4
3.65	114.4
3.70	104.6
3.75	96.4
3.80	90.2
3.85	84.6
3.90	79.6
3.95	74.7
4.00	71.4

Titration No. 7

vol. added (ml.)	e.m.f. (mV)
6.00	174.7
6.40	163.6
6.60	156.8
6.80	149.4
7.00	141.5
7.20	133.7
7.40	126.6
7.50	123.2
7.60	120.1
7.80	114.4
8.00	109.4
8.20	104.8
8.40	100.7
8.60	96.9
8.80	93.3
9.00	90.0
9.25	86.1
9.50	82.6
9.75	79.1
10.00	75.9

Titration No. 8

vol. added (ml.)	e.m.f. (mV)
6.50	167.4
6.60	161.8
6.70	155.4
6.80	148.5
7.00	134.6
7.10	128.1
7.20	122.5

Titration No. 8 contd.

vol. added (ml.)	e.m.f. (mV)
7.30	117.3
7.40	112.6
7.60	104.9
7.80	98.2
8.00	92.6
8.20	87.5
8.60	78.8
9.20	68.1
9.60	61.9
10.00	56.1

Titration No. 9

vol. added (ml.)	e.m.f. (mV)
6.60	167.6
6.70	157.3
6.80	144.9
6.90	132.6
7.00	122.1
7.10	113.3
7.20	106.1
7.30	100.0
7.40	94.8
7.60	86.1
7.80	79.0
8.00	72.8
8.40	62.2
8.80	53.3

Titration No. 10

vol. added (ml.)	e.m.f. (mV)
3.20	177.1

Titration No. 10 contd.

vol. added (ml.)	e.m.f. (mV)
3.40	167.7
3.60	157.3
3.80	147.3
4.00	138.2
4.20	130.2
4.40	123.4
4.60	117.2
4.80	111.7
5.00	106.6
5.20	101.8
5.60	93.2
6.00	85.2
6.80	70.1
7.20	62.7
7.60	55.3
8.00	47.7

Titration No. 11

vol. added (ml.)	e.m.f. (mV)
3.40	171.1
3.50	161.9
3.60	152.4
3.70	143.4
3.80	135.8
4.00	123.4
4.20	113.3
4.40	105.4
4.80	92.2
5.20	81.2
5.60	71.4

Titration No. 11 contd.

vol. added (ml.)	e.m.f. (mV)
6.00	62.0

Titration No. 12

vol. added (ml.)	e.m.f. (mV)
3.40	164.4
3.50	146.4
3.60	132.4
3.65	126.9
3.70	121.5
3.80	113.2
4.00	100.1
4.20	89.9
4.40	81.0

Titration No. 13

vol. added (ml.)	e.m.f. (mV)
5.20	190.9
5.30	182.8
5.40	173.3
5.50	163.1
5.60	153.4
5.70	145.0
5.80	137.6
5.90	131.3
6.00	125.7
6.20	116.6
6.40	108.9
6.80	96.4
7.20	85.7

Titration No. 14

vol. added (ml.)	e.m.f. (mV)
21.60	154.2
22.40	149.6
23.20	145.5
24.00	141.7
24.80	138.3
25.60	135.1
26.40	132.1
27.20	129.3
28.00	126.6
28.80	123.9
29.60	121.4
30.00	120.0
32.00	114.8
34.00	109.2
36.00	103.5
38.00	97.9
39.00	95.2
40.00	92.0
42.00	86.6
44.00	80.2
45.00	76.8
46.00	73.1
47.00	69.3
48.00	65.1

Titration No. 15

vol. added (ml.)	e.m.f. (mV)
3.60	-28.5
4.00	-22.1
4.40	-14.4

Titration No. 15 contd.

vol. added (ml.)	e.m.f. (mV)	vol. added (ml.)	e.m.f. (mV)
4.80	- 4.6	7.20	81.3
5.00	1.6	7.60	88.3
5.20	8.8	8.00	94.1
5.40	17.1	8.80	104.0
5.60	26.9	9.60	112.1
5.70	32.1	10.00	116.1
5.90	42.4	12.00	132.6
6.20	54.9	14.00	147.5
6.40	61.8	16.00	162.7
6.60	67.9	18.00	177.9
6.80	72.9		

The formation curves were coincident, as represented in Figure 5, indicating the presence of simple A_nB species and absence of polynuclear, hydrolysed and protonated complexes. The analysis of the data using SCOGS gave the following results :-

$$\log\beta_{110} = 5.070 \pm 0.004$$

$$\log\beta_{210} = 9.426 \pm 0.004$$

$$\log\beta_{310} = 12.300 \pm 0.026$$

s(228 readings) in titre = 0.104

A search for possible protonated species in the low $\bar{Z}$ region of the formation curve proved negative.

Formation constants for copper(II) - asparaginate complexes.

The hydrogen ion concentration in the vessel was varied by titrating alkali into a mixture of ligand, metal ion, and mineral acid.

Table 6 Experimental results for the Cu(II)-asparaginate system

Titration number	Initial concentrations (mM) Cu(II)	ligand	acid	Initial volume (ml)	E^o (mV)
1	2.958	3.031	2.495	24.97	415.8
2	9.484	37.92	8.002	24.97	417.7
3	9.478	19.07	7.997	24.97	417.0
4	9.478	9.471	7.997	24.97	414.2
5	18.91	75.85	15.95	24.97	416.2
6	18.82	39.75	15.87	24.97	416.6
7	19.10	18.88	16.11	24.97	416.6
8	47.39	168.9	39.99	24.97	414.2
9	47.39	116.2	39.99	24.97	415.4
10	47.39	49.37	39.99	24.97	416.5

The concentration of the titrant alkali was 79.90mM in titrations 1-4 and 200.1mM in Nos. 5-10.

Titration No. 1

vol. added (ml.)	e.m.f. (mV)
0.00	254.1
0.40	243.7
0.80	229.7
1.10	215.5
1.30	203.5

Titration No. 2

vol. added (ml.)	e.m.f. (mV)
0.00	256.5
0.40	254.3
0.80	252.0
1.20	249.6
2.00	244.4
2.80	240.5
3.60	234.3
4.40	227.3
5.20	219.1
6.00	209.5
6.80	197.6
7.20	190.6
7.60	182.6
8.00	172.9

Titration No. 3

vol. added (ml.)	e.m.f. (mV)
0.80	260.4
1.60	254.2
2.40	247.6
2.80	244.0
3.20	240.2
3.60	236.1

Titration No. 4

vol. added (ml.)	e.m.f. (mV)
0.00	277.0
0.20	275.0
0.40	273.0
0.60	271.0
0.80	269.0
1.20	264.5
1.40	262.2
1.60	259.7
1.80	257.3
2.05	254.2
2.30	250.9
2.60	246.7
2.90	242.2
3.20	237.5

Titration No. 5

vol. added (ml.)	e.m.f. (mV)
0.0	259.5
0.40	257.7
1.20	251.7
2.00	245.4
2.40	241.9
2.80	238.2
3.20	234.3
3.60	230.1
4.00	225.4
4.40	220.4
4.80	214.9
5.20	208.5
5.60	201.3

Titration No. 5 contd.

vol. added (ml.)	e.m.f. (mV)
6.00	192.6
6.40	187.5

Titration No. 6

vol. added (ml.)	e.m.f. (mV)
1.20	262.6
1.80	255.8
3.20	237.5
4.00	223.7
4.40	215.2
4.80	205.1
5.20	193.2
5.40	186.2
5.60	178.7
5.80	170.3
6.00	160.9
6.20	150.4
6.40	138.0
6.50	130.9

Titration No. 7

vol. added (ml.)	e.m.f. (mV)
0.00	289.2
0.40	285.3
0.80	279.6
1.20	273.5
1.60	267.2
2.00	260.6
2.40	253.7
2.80	246.4

Titration No. 8

vol. added (ml.)	e.m.f. (mV)
0.00	269.0
0.80	266.6
1.60	264.0
2.40	261.2
3.20	258.4
4.00	255.5
4.80	252.6
5.60	249.5
6.40	246.4
7.20	243.0
8.00	239.5
8.80	235.8
9.60	231.9
10.40	227.7
11.20	223.1
12.00	218.1
12.80	212.7
13.60	206.4
14.40	199.4
15.20	191.0
16.00	180.6
16.40	174.2

Titration No. 9

vol. added (ml.)	e.m.f. (mV)
0.00	280.8
0.80	278.3
1.60	275.1
2.40	271.8
3.20	268.5
4.00	265.1

Titration No. 9 contd.

vol. added (ml.)	e.m.f. (mV)
4.80	261.7
5.60	258.1
6.40	254.4
7.20	250.5
8.00	246.4
8.80	242.0
9.60	237.3
10.40	233.3
11.20	228.0
12.00	222.0
12.80	215.3
13.60	207.7
14.40	199.0
15.20	188.7

Titration No. 10

vol. added (ml.)	e.m.f. (mV)
0.00	308.3
0.80	303.3
1.60	279.2
2.40	291.2
3.20	285.5
4.00	279.7
4.80	274.1
5.60	268.3
6.40	262.3
7.20	256.0
8.00	241.4

Figure 5 shows that the curves do not coincide as $\bar{Z}$ approaches 2.0. The experimental data were processed in SCOGS and an extensive survey of possible species was made, including reference to hydrolysed species which have been previously reported[28,135]. For $A_pB_qH_r$, species with the values pqr 110, 210, 310, 111, 211, 212, 11-1 and 22-2 were searched for, but in the end only the simple binary species 110 and 210 were found. The constants were:-

$$\log \beta_{110} = 8.677 \pm 0.023$$

$$\log \beta_{210} = 16.052 \pm 0.024$$

$$s(128 \text{ readings}) = 0.294$$

The copper-asparaginate results were selected to illustrate the effect of various experimental errors on the constants.

Table 7. Effect of experimental errors

Variable and Variation	$\log \beta_{110}$	difference
Increase T by $0.25^{o}C$	8.703	+ 0.026
Increase initial volume by 1%	8.667	- 0.010
Increase $[H^+]$ by 1%	8.660	- 0.017
Increase [A] by 1%	8.670	- 0.007
Increase [B] by 1%	8.678	+ 0.001
Increase E^o by 1mV	8.625	- 0.052

It is at once evident that E^o is the most sensitive variable and so care must be exercised in its determination.

Formation constants for nickel(II)-asparaginate complexes.

This system was studied before Zn and Cu, and therefore has the most extensive series of titration conditions.

(a) Constant metal ion and constant ligand concentrations-acid titrated into alkali (titrations 1-3).

(b) Constant ligand concentration only - alkali into acid (titration 5 and 8).

(c) Metal ion, mineral acid and ligand titrated with alkali (titrations 4, 6, 9-10).

(d) Ligand and alkali titrated with metal ion and mineral acid (titrations 7, 11-14).

(e) Metal ion and mineral acid titrated with ligand and alkali (titration 16).

(a) is the method favoured by the Sillén school. It keeps metal ion and ligand constant, but the disadvantage of this is that a relatively narrow pH range is covered. Two series of solutions with ligand: metal ion ratio 4:1, 2:1, 1:1 etc. are made up, one containing mineral acid and the other alkali, each successive member of the series being prepared from the previous one by dilution with a metal ion solution which is itself necessarily acid to prevent hydrolysis. Therefore each member of the alkaline series becomes progressively less alkaline and the pH range narrows.

(b) overcomes this drawback, but is wasteful on ligand. The methods normally used are (c), (d) and (e) with (c) being the most popular.

Table 8. Experimental results for the Ni(II)-asparaginate system

Titration No.	Initial concentrations in S*(mM)			Initial concentrations in T*(mM)			Initial volume (ml)	E^o (mV)
	Ni(II)	ligand	mineral acid.	Ni(II)	ligand	mineral acid.		
1	1.931	8.096	- 3.797	1.931	8.096	6.400	19.98	408.0
2	4.830	20.00	- 8.900	4.830	20.00	8.100	19.98	408.0
3	1.894	7.833	- 7.284	1.931	7.979	32.49	24.97	408.0
4	4.830	10.00	5.500	0.0	0.0	- 25.00	24.97	412.8
5	9.876	20.21	57.50	0.0	20.04	- 100.0	19.98	412.8
6	19.31	39.9	21.50	0.0	0.0	- 60.00	24.97	412.8
7	0.0	19.79	- 4.000	48.30	0.0	30.10	24.98	412.8
8	38.64	119.7	77.73	0.0	120.5	- 220.00	19.98	412.8
9	4.830	5.000	43.52	0.0	0.0	- 25.00	24.97	406.5
10	19.31	19.95	17.40	0.0	0.0	- 60.00	24.97	406.5
11	0.0	166.8	- 20.00	48.30	0.0	30.10	24.97	437.8
12	0.0	166.8	- 20.00	96.46	0.0	60.20	24.97	435.3
13	0.0	83.43	- 10.00	48.30	0.0	30.10	24.97	437.6
14	0.0	83.43	- 10.00	24.14	0.0	15.05	24.97	436.1
15	24.14	0.0	15.05	0.0	166.8	- 20.00	19.98	435.0

* S refers to initial solution in the vessel (titrate)
T refers to the solution being added from the burette (titrant)
Negative acid concentrations refer to alkali

Titration No. 1

vol. added (ml.)	e.m.f. (mV)
0.00	-29.0
0.80	-7.9
1.20	1.4
2.40	22.6
2.80	28.8
3.60	40.2
4.00	45.6
4.80	55.7
5.00	62.6

Titration No. 2

0.00	0.5
0.80	15.4
2.00	32.1
3.00	43.5
4.00	53.5
5.00	62.4
20.00	154.4
22.00	162.9
24.00	170.7
27.00	180.9
30.00	188.9

Titration No. 3

13.60	38.6
15.00	53.2
18.00	75.2
20.00	86.4
21.00	91.4

Titration No. 3 contd.

vol. added (ml.)	e.m.f. (mV)
25.00	108.5

Titration No. 4

4.40	205.2
5.20	188.0
6.00	156.8
6.80	124.9
7.60	103.1
8.40	86.4
9.20	71.9
9.60	65.4
10.50	46.5

Titration No. 5

12.80	108.9
13.60	78.6
14.40	48.3
15.20	8.8

Titration No. 6

1.20	252.2
2.00	248.6
2.80	244.3
4.00	237.4
4.40	235.0
6.00	223.9
6.80	217.2
7.60	209.1
8.40	199.2

Titration No. 6 contd.

vol. added (ml.)	e.m.f. (mV)
9.20	187.2
10.00	172.9
10.80	163.8
11.60	151.6
12.40	140.0
12.80	134.8

Titration No. 7

0.70	-20.0
0.80	21.0
0.90	44.5
1.10	71.8
1.30	89.2
1.50	102.2
1.80	117.8
2.00	126.6
2.50	145.8
3.50	177.8
4.00	190.1
4.50	199.7
5.00	207.2

Titration No. 8

5.60	215.8
6.40	203.9
7.20	189.7
8.80	161.4

Titration No. 9

Titration No. 9

vol. added (ml.)	e.m.f. (mV)
4.40	144.7
5.20	104.1
5.60	91.6
6.00	80.0
6.80	57.7
7.20	46.6
7.60	35.3
8.00	22.6
8.80	-12.1

Titration No. 10

vol. added (ml.)	e.m.f. (mV)
3.00	246.3
4.00	237.0
5.00	225.8
6.00	210.3
7.00	185.9
8.00	154.7
9.00	130.8
10.00	112.6
11.00	97.6
11.40	91.8
11.80	85.8
12.20	79.6
12.60	73.1
13.00	66.4

Titration No. 11

vol. added (ml.)	e.m.f. (mV)
2.30	-18.9
2.40	-15.2

Titration No. 10 contd.

vol. added (ml.)	e.m.f. (mV)
2.50	-11.3
2.60	-7.2
2.70	-2.6
2.80	2.4
2.90	7.5
3.00	13.3
3.10	19.2
3.20	25.6
3.30	32.6
3.40	40.7
3.50	49.8
3.60	59.6
3.70	69.8
3.80	79.7
4.00	95.5
4.10	101.7
4.20	107.1
4.40	115.7
4.60	122.8
4.80	128.8
5.00	133.9
5.20	138.4
5.40	142.5
5.60	146.2
6.00	152.6
6.40	158.1
6.80	162.8
7.20	167.0
7.60	170.8

Titration No. 11 contd.

vol. added (ml.)	e.m.f. (mV)
8.00	174.3
8.40	177.4
8.80	180.3
9.20	183.0
9.60	185.6
10.00	187.9
11.00	193.2
12.00	197.8
13.00	201.9
14.00	205.7
15.00	209.1
17.00	215.1
18.50	219.3
20.00	223.0

Titration No. 12

vol. added (ml.)	e.m.f. (mV)
1.20	-16.4
1.30	-8.0
1.40	2.5
1.50	13.3
1.60	26.7
1.70	42.6
1.80	62.1
1.90	82.6
2.00	97.7
2.10	109.1
2.20	117.6
2.30	124.4
2.40	130.3

Titration No. 12 contd.

vol. added (ml.)	e.m.f. (mV)
2.60	139.9
2.80	147.5
3.00	153.9
3.20	159.3
3.60	168.3
4.00	175.4
4.40	181.5
4.80	186.7
5.20	191.2
5.60	195.3
6.00	198.9
7.00	206.6
8.00	213.2
9.00	218.9

Titration No. 13

3.20	151.0
3.60	159.9
4.00	167.4
4.40	173.7
4.80	179.1
5.50	187.2
6.50	196.6
8.00	207.8

Titration No. 14

7.00	152.5
7.50	157.6
8.00	162.2

Titration No. 14 contd.

vol. added (ml.)	e.m.f. (mV)
8.50	166.2
9.00	170.0
9.50	173.4
10.00	176.6
11.00	182.2
12.00	187.1
14.00	196.7
16.00	203.2

Titration No. 15

3.00	279.0
4.00	267.5
5.00	258.3
6.00	250.6
7.00	243.8
8.00	237.7
9.00	232.2
10.00	227.4
12.00	218.7
14.00	211.4
16.00	204.9
18.00	199.2
20.00	194.4
22.00	189.2
24.00	184.7
26.00	180.4
28.00	176.3
30.00	172.1
32.00	168.2

Titration No. 15 contd.

vol. added (ml.)	e.m.f. (mV)
36.00	160.2
40.00	156.2

As the formation curves (figure 5) coincide the system is described by simple A_nB species. The data was analysed using SCOGS and the following results were obtained.

$$\log \beta_{110} = 6.152 \pm 0.007$$

$$\log \beta_{210} = 11.163 \pm 0.011$$

$$\log \beta_{310} = 14.545 \pm 0.055$$

s (206 readings) in titre = 0.244.

Formation constants for Co(II), Fe(II) and Mn(II)-asparaginate complexes

The formation constants for the complexes between asparaginate and manganese(II), iron(II) and cobalt(II) had previously been measured in this laboratory,[136] and they are listed in table 9. The formation curves can all be found in figure 5.

Table 9. Formation constants for Co(II), Fe(II) and Mn(II)-asparaginate complexes

Metal ion	$\log \beta_{110}$	$\log \beta_{210}$	$\log \beta_{310}$	number of readings	s in titre
Mn(II)	3.102 ± 0.040	5.222 ± 0.090		46	0.310
Fe(II)	4.366 ± 0.033	7.569 ± 0.036	10.259 ± 0.054	56	0.215
Co(II)	4.903 ± 0.007	9.029 ± 0.011	11.855 ± 0.021	60	0.120

Comparison with other workers' results.

Work on metal-asparaginate complexation is considerably less extensive than other amino-acids. However, comparison between results in this work and those obtained by other workers reveals that the formation constants obtained in 3.00M $(Na)ClO_4$ are higher than those obtained at lower ionic strengths, a trend which

has been observed previously[29,134].

Previous work on the Mn(II), Fe(II) and Zn(II)-asparaginate systems is very scanty and the constants which have been obtained are not well characterised. The only A_3B complex to be reported outside the present work is that of cobalt[30], which appears, together with the other reported results, in Table 10.

Table 10 Literature values for metal ion-asparaginate complex formation constants

Metal Ion	Temp. °C	Method	I ionic background	$\log \beta_{110}$	$\log \beta_{210}$	$\log \beta_{310}$	Reference
Zn(II)	25	gl	3.00M	5.070	9.426	12.300	This work
	20	gl	0.01M		8.7		70
	15	gl	0.005M		8.5		71
Cu(II)	25	gl	3.00M	8.677	16.052	not formed	This work
	25	gl	0.20M	7.79	14.29		137*
	25	gl	0.16M	7.78	14.13		138
	25	gl	0.10M	7.86	14.42		31
	20	gl	0.01M		14.9		70
Ni(II)	25	gl	3.00M	6.152	11.163	14.545	This work
	25	gl	0.16M	5.58	9.96		138
	25	gl	0.10M	5.68	10.23		31
	20	gl	0.01M		10.6		70
Co(II)	25	gl	3.00M	4.903	9.029	11.855	136
	25.15	gl	0.15M	4.55	8.13	9.95	30
	25	gl	0.10M	4.51	8.01		31
	20	gl	0.01M		8.4		70
Fe(II)	25	gl	3.00M	4.366	7.569	10.259	136
	20	gl	0.01M		6.5		70
Mn(II)	25	gl	3.00M	3.102	5.222	not formed	136
	20	gl	0.01M		4.5		70

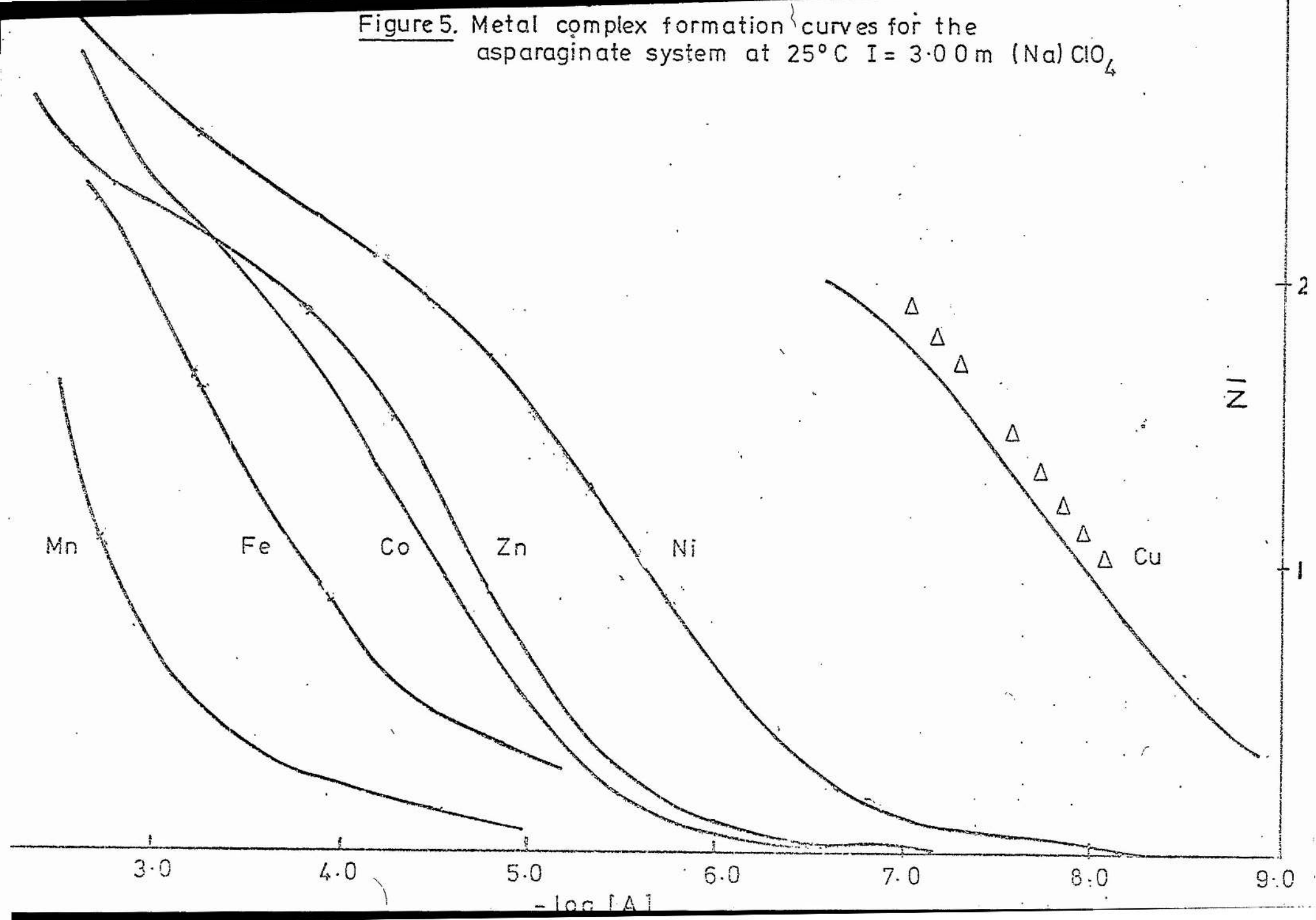
gl = glass electrode measurement

* In addition, Gergely et al[137] obtained constants for the following equilibria:-

$$BA_2H_{-1} + H^+ \rightleftharpoons BA_2, \log K = 10.45$$

$$BA_2H_{-2} + H^+ \rightleftharpoons BA_2H_{-1}, \log K = 12.0$$

Figure 5. Metal complex formation curves for the asparaginate system at 25° C I = 3·00 m (Na) ClO_4



CHAPTER 6. CALORIMETRY

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Chapter 6. CALORIMETRY

Description of apparatus.

The calorimeter was of the Gerding, Leden and Sunner design[139] and had previously been used to determine heats of formation for other metal ion-amino-acid complexes in this laboratory[27-28]. The unit consisted of an inner reaction vessel of glass (capacity ca 150ml) and an outer shielding vessel of copper, nickel plated externally.

The two vessels were attached to the "lid" of the calorimeter, the reaction vessel by two springs and the outer vessel by an O-ring seal to prevent leakage. Chimneys provided the means of introducing the following probes into the reaction vessel : (a) a burette tip (b) a stirrer which also acted as a cooler, (c) a heat detector, (d) a heater, (e) an electrode pair.

(a) The burette : The titrant was added through a glass burette tip and could be protected from back diffusion by a polyfluorotetraethylene (PTFE) cap held on a gold spring. The titrant was prewarmed to the bath temperature in a spiral of nylon tube (Porlex SFD Nylon C, ca 8ml capacity) on top of the vessel, by immersion in the bath. The free end of the nylon tube was then connected to a piston burette (Metrohm AG, E274).

(b) The stirrer : Vibro-stirring was used because it has smaller heat of stirring corrections and is more efficient than paddle-stirring, especially in eliminating localised precipitation around the burette tip on addition of titrant. Such precipitates not only give rise to spurious enthalpy effects but are often slow to redissolve. The stirrer disc was a flat circular plate

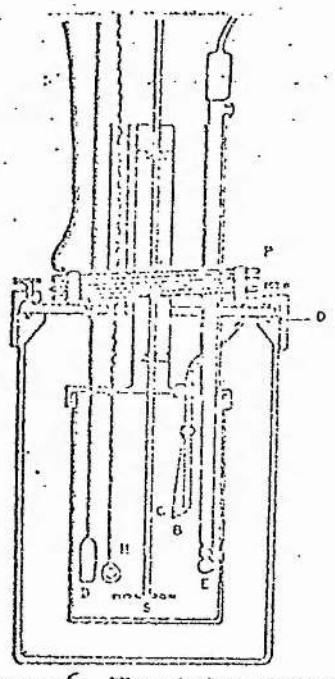

FIGURE 6 The solution calorimeter

B- Burette
C - PTFE cap
D - Heat detector
E - Combination electrode
H - Heater
O - O-ring seal
P - Prewarming spiral
S - Stirrer

of 1mm PTFE (2.5cm diameter) containing ten holes of 1mm diameter. This was screwed onto a hollow stainless steel tube suspended by two ball races and having a rubber sealing diaphragm on the bottom of the centre chimney.

The connection from the vibro-motor (Chemap AP, E1) to the calorimeter was an L-shaped bar which was in two pieces, the shorter (10-12cm), nearer the calorimeter, sliding into the longer piece and held in place by a screw through both pieces. The other end of the shorter piece had two prongs which fitted round a rubber grommet near the top of the stirrer tube. This arrangement prevented vibrations from the motor being transmitted to the calorimeter unit as a whole. The stirrer tube was hollow to allow drops of liquid nitrogen to be passed into it from a long hollow needle.

(c) Heat detector : The temperature change upon addition of titrant to the reaction vessel was measured by use of a quartz thermometer (Hewlett Packard 2801A) coupled to a digital recorder (Hewlett Packard 562A) which printed out temperature readings correct to 0.0001^{o} at intervals of 18 seconds. Provided the entire system was well equilibrated at $25.00^{0}C$, i.e. that it had been immersed in the bath since the previous evening, and the calorimetry room was maintained at $22^{o}C$ these readings, when plotted, gave "fore" and "aft" periods which were parallel and so the reaction heats could quite easily be expressed as $\Delta^{o}K$.

(d) Calibration heater : To convert change in temperature, $\Delta^{o}K$, to energy required a calibration experiment using a known quantity of heat.

This was done electrically by means of a heater coil of non-inductively wound resistance wire (22.0 Ω), coated with a chemically-resistant epoxy resin (Araldite). The voltage across the heater was measured on a digital voltmeter (Solartron LM 1420.2). The current flowing was passed through the heater resistor and also through a 10.00 Ω standard resistance: the measurement of the current passing through this resistance gave the current in the heater circuit. The time for which the heating current flowed was automatically recorded to within 0.02 seconds using a stop-watch (Jaquet 309e) coupled to the "on-off" switch controlling the calibration heater.

(e) Electrode Pair : Although there is provision for the use of a combination electrode (Activion T1N7DR/180), it was not used in this work as more accurate results can be obtained by direct potentiometry on the bench.

The complete system was immersed in a thermostat bath controlled to 25.0000 $\pm$ 0.0005°C (LKB 7602 controller on 7603A bath) which was located in a thermostatted room (22.0 $\pm$ 0.5°C).

Experimental procedure.

For each titration the vessel was charged by directly pipetting the titrate into the reaction vessel or making up the titrate in a siliconed flask and pouring the contents (99.57ml) into the reaction vessel. The calorimeter was assembled and immersed in the water bath. After a minimum of eight hours (usually overnight) the stirring and electronic systems were switched on, and two hours later reached steady state

conditions when the titration could proceed.

For each point the following procedure was adopted.

(1) 12-minute "fore" period.

(2) 6-minute reaction period in which titrant was added (or electrical energy for a calibration point).

(3) 12-minute "aft" period.

(4) Nitrogen cooling, if required.

In many cases step (4) was not required and the "aft" period of one point became the "fore" period of the next point.

The values of 12, 6 and 12 minutes were arrived at by plots of temperature against time, and the above values were the times required to reach steady state.

Figure 7. Temperature-time plot for calorimeter reaction period.

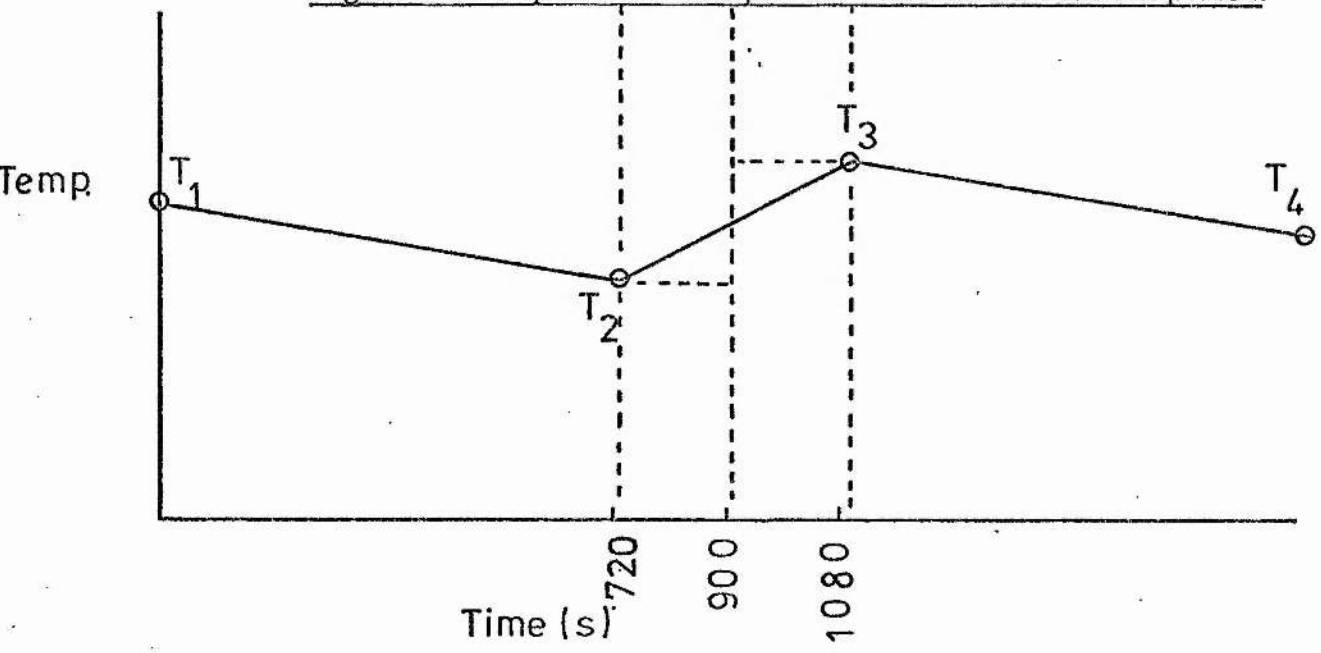

Figure 7. Temperature-time plot for calorimeter reaction perio

The heat corrections were calculated from the following equation:

$$\Delta T = T_3 + \frac{(T_3 - T_4)}{4} - T_2 - \frac{(T_1 - T_2)}{4}$$

The reference point for calculation of the temperature change was the mid-point of the reaction period, i.e. three minutes after the addition of titrant. This point was chosen from the temperature-time plot, and is the method originally described by White[140].

Calibrations were performed during the course of titrations and a body of data was built up to construct a calibration line. For each calibration point electrical energy was applied to the system for 60 seconds and the energy was calculated from :

$$J = Vit$$

where J is in joules, V in volts, i in amps and t in seconds. The calibration constant was evaluated as :

$$x = \frac{J}{\Delta T}$$

and was plotted against the total volume of solution in the reaction vessel.

Heat of formation of water, ΔH°_{W}

This very important quantity was determined to assess the accuracy and reproducibility of the calorimeter. As in subsequent experiments, alkali was titrated into acid, and the results obtained, for formation of 2.0×10^{-4} moles of water, were as follows :

(i) -55.740 kJ mol^{-1} (-13.322 kcal mol^{-1})

(ii) -55.886 kJ mol^{-1} (-13.357 kcal mol^{-1})

(iii) -55.693 kJ mol^{-1} (-13.310 kcal mol^{-1})

mean value -55.773 ± 0.075 kJ mol^{-1} (-13.330 ± 0.018 kcal mol^{-1})

This value agrees well with the literature, where the "best" calorimetric value is given as "close to -13.34 kcal mol^{-1}"[141].

Heats of protonation for asparaginate.

The heats of protonation for asparaginate in 3.00M $(Na)ClO_4$ had been measured previously in this laboratory[134], and the results were as follows :

$-NH_2$ group	-50.5 ± 0.4 kJ mol^{-1}
$-CO_2^-$ group	-1.5 ± 3.5 kJ mol^{-1}
	-5.10 ± 0.005 kJ mol^{-1} by microcalorimetry.

The latter value was used in subsequent calculations.

Heats of formation for Zn(II)-asparaginate complexes.

These were measured by titrating sodium hydroxide (125.0mM) into Zn(II)-asparaginate solutions. In all cases the initial volume was 100.0ml.

Table 11. Calorimetric results for the Zn(II)-asparaginate system.

Initial [Zn(II)] mM	4.707	7.079	7.079
Initial [asn] mM	14.16	22.82	16.92
Initial [H] mM	18.61	29.51	23.61
Volume added (ml)	heat evolved (joules)		
4.0		(9.220)	(4.460)
5.0	1.981		(3.665)
6.0	2.101	(7.253)	2.451
7.0	1.869		2.323
8.0	1.887	3.927	2.091
9.0	2.111		
10.0		4.366	4.158
12.0		3.918	4.289
14.0		4.476	4.476
16.0		4.227	3.294
18.0		3.800	2.347
20.0		2.558	

The values of ΔH_1^o, ΔH_2^o and ΔH_3^o were calculated by the computer program RWCALCRD containing least squares program RWSOLV.

The results were :

$$\Delta H_1^o = -10.44 \pm 0.40 \text{ kJ mol}^{-1}$$

$$\Delta H_2^o = -23.17 \pm 0.80 \text{ kJ mol}^{-1}$$

$$\Delta H_3^o = -27.55 \pm 1.20 \text{ kJ mol}^{-1}$$

s(19 readings) = 0.40

The enthalpic curve is shown in figure 8.

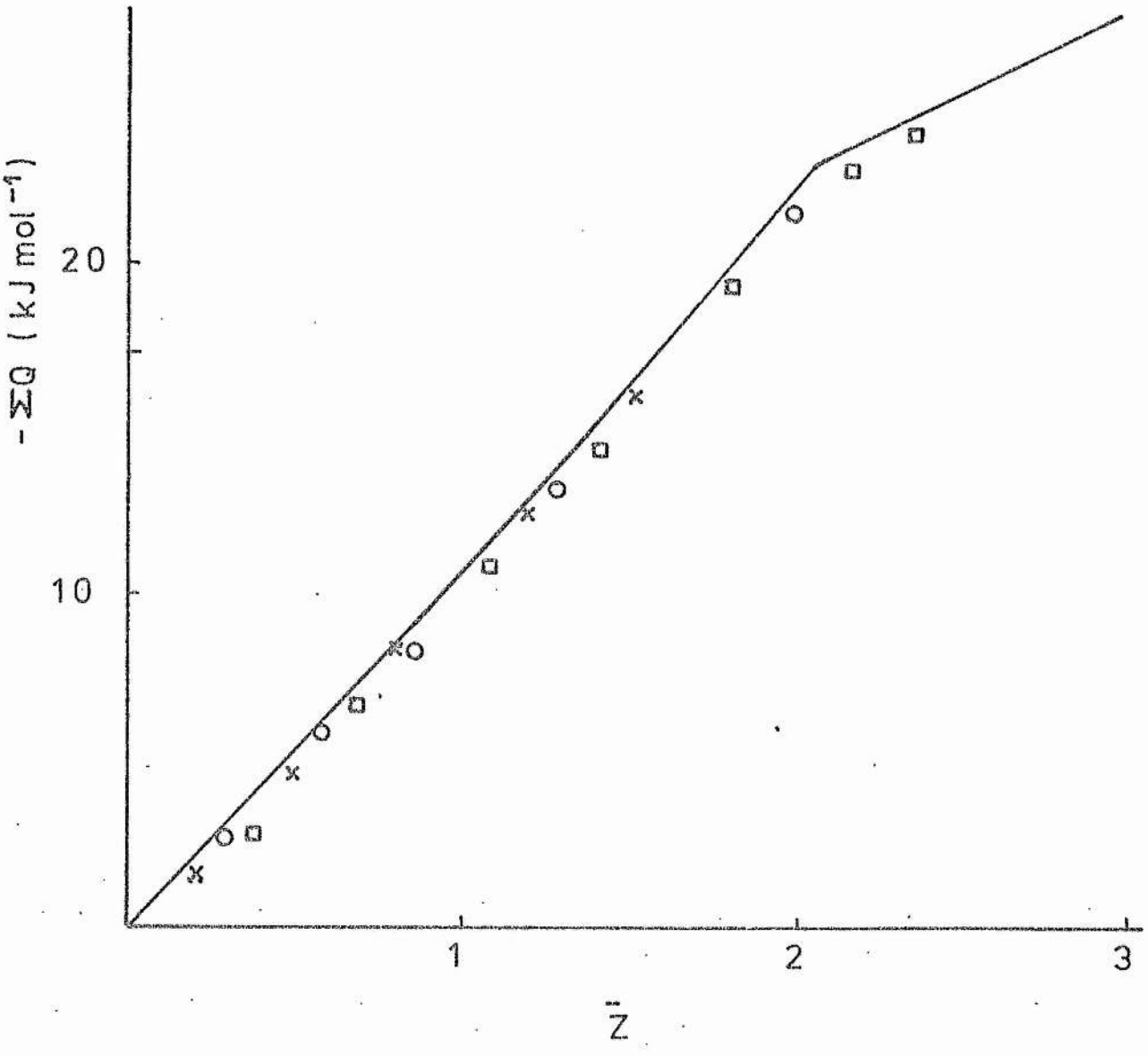

Figure 8 Enthalpic curve for the Zn (II)-Asparaginate system.

Note on enthalpic curves An enthalpic curve is a graph of total heat evolved against $\bar{Z}$, and can be drawn from output data generated by RWCALCRD. The solid line on each of these graphs, going through the origin of the axes, is the theoretical curve. The experimental curves for mononuclear complexes are parallel to the theoretical curve, and are superimposable upon it after each titration has been corrected for its initial $\bar{Z}$. Since a negative $\bar{Z}$ has no meaning, all the experimental curves should lie below the theoretical curve. As will be seen shortly, in the case of the copper(II) titrations the initial $\bar{Z}$ was often 0.30 or greater, hence producing an experimental curve some distance from the theoretical curve.

At the commencement of each titration the pH was between 2 and 3 so for the first few points in each titration the heat given out was that due to neutralisation of excess mineral acid (present to prevent hydrolysis) by alkali, and no significant complexation occurred until pH 4 or 5. When the heats for these early points was corrected for formation of water and protonation of the ligand, they had negative values and were therefore discarded. These discarded values are shown in brackets in the tables.

Heats of formation for copper(II)-asparaginate complexes.

These were measured by titrating sodium hydroxide (125.0mM) into Cu(II)-asparaginate solutions. The initial volume was 100.0ml in each titration.

Table 12. Calorimetric results for the Cu(II)-asparaginate system.

Initial [Cu(II)] mM	7.139	4.756	9.503
Initial [asn] mM	14.47	14.46	14.43
Initial [H] mM	20.49	18.48	22.45
volume added (ml)	heat evolved (joules)		
1.0		4.663	
2.0	9.517	4.855	9.517
3.0		4.564	
4.0	9.563	4.463	9.612
5.0		4.557	
6.0	9.954	4.952	9.854
7.0		5.101	
8.0	10.556	3.927	10.709
9.0		4.170	
10.0	10.134	4.470	10.186
11.0		4.039	
12.0	8.895		9.583
14.0	9.113		9.814
16.0	9.333		9.498
18.0			9.108
20.0			7.334

When the results of these titrations came to be processed by the previous method, difficulties were encountered. With both AB and A_2B complexes taken into consideration, RWSOLV gave excessively high values for the heats, and when only the 1:1 complex was considered the $\triangle H^o$ value obtained was of the right order of magnitude but had the wrong sign.

The $\log \beta$ values for the Cu(II)-asparaginate complexes were

considerably larger than those for any of the other metal ions under examination, and in all the Cu(II)-asparaginate titrations there was some degree of complexing even at $-\log h = 2.5$, whereas in other systems comparable complexation did not occur until $-\log h = 5.0$.

As can be seen, the experimental enthalpic curves lie a considerable distance from the theoretical curve which is drawn with the assumption that $\bar{Z} = 0.0$ at the commencement of a titration. The initial values of $\bar{Z}$ in the copper(II)-asparaginate titrations were 0.37, 0.52 and 0.32 respectively.

The ΔH^{o} values were calculated by hand, and the results obtained were as follows.

$$\Delta H_1^o = -27.5 \pm 1.0 \text{ kJ mol}^{-1}$$

$$\Delta H_2^o = -61.5 \pm 2.0 \text{ kJ mol}^{-1}$$

$$s(29 \text{ readings}) = 1.0$$

The enthalpic curve is shown in Figure 9.

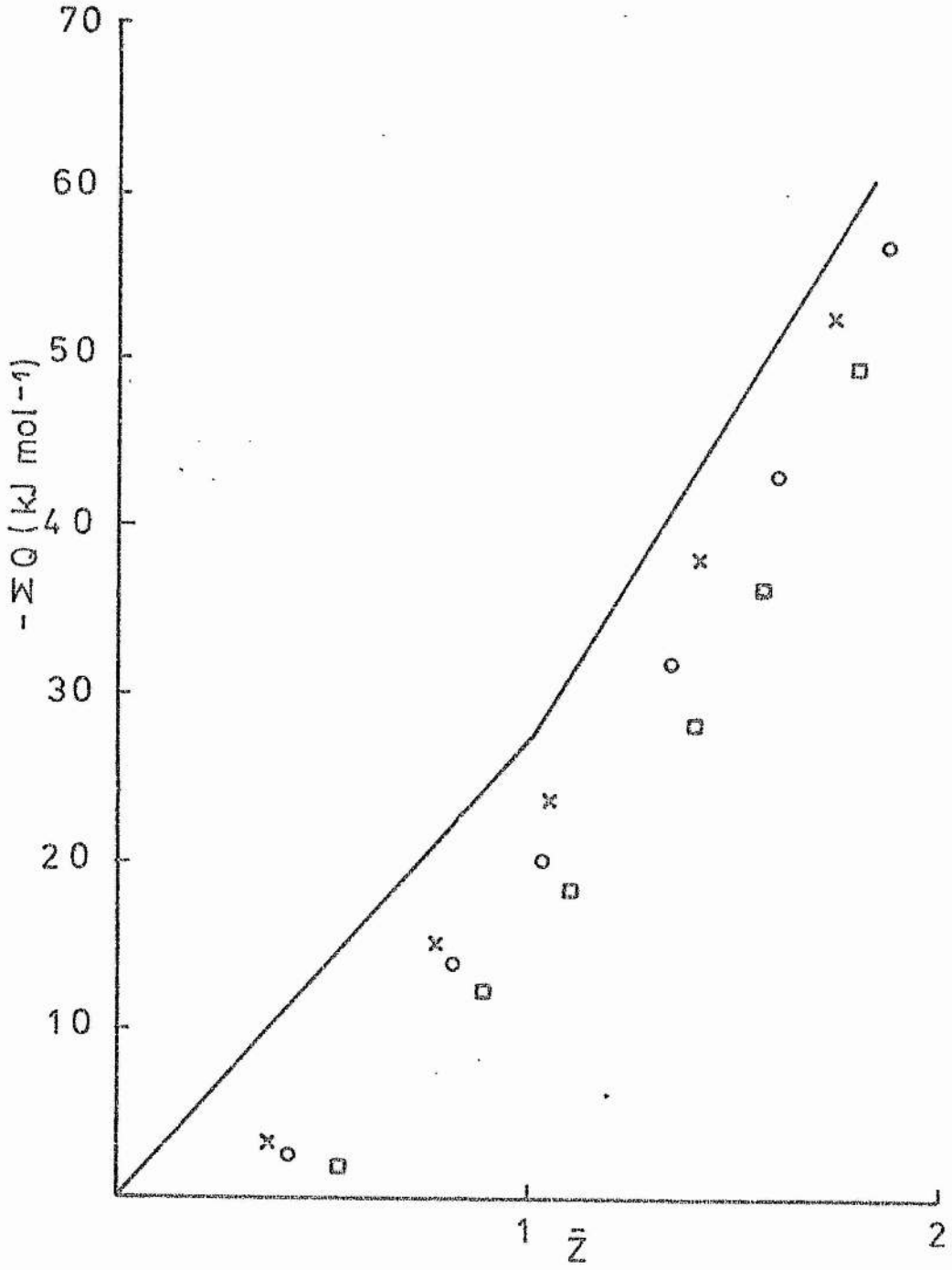

Figure 9. Enthalpic curve for the Cu (II)-Asparaginate system.

Heats of formation for nickel(II)-asparaginate complexes.

These were obtained by titrating sodium hydroxide (99.96mM) into nickel(II)-asparaginate solutions. The initial volume was 100.0ml.

Table 13. Calorimetric results for the Ni(II)-asparaginate system.

Initial [Ni(II)] mM	4.838	4.848	7.277
Initial [asn] mM	14.26	7.733	28.63
Initial [H] mM	17.28	10.75	33.16
volume added (ml)	heat evolved (joules)		
4.0	6.179	2.501	
5.0		2.675	
6.0	4.542	2.651	3.001
7.0		2.525	2.778
8.0	5.711	2.499	2.703
9.0		2.935	2.883
10.0	5.925	2.599	2.391
11.0		1.417	2.833
12.0	5.612		3.018
13.0			2.672
14.0	5.392		2.642
16.0	4.337		5.874
18.0	1.602		6.202
20.0			5.742

The values of ΔH_1^o, ΔH_2^o and ΔH_3^o calculated from RWCALCRD and RWSOLV were as follows :

$$\Delta H_1^o = -17.11 \pm 0.40 \text{ kJ mol}^{-1}$$

$$\Delta H_2^o = -43.45 \pm 0.80 \text{ kJ mol}^{-1}$$

$$\Delta H_3^o = -63.00 \pm 1.20 \text{ kJ mol}^{-1}$$

s(28 readings) = 0.40

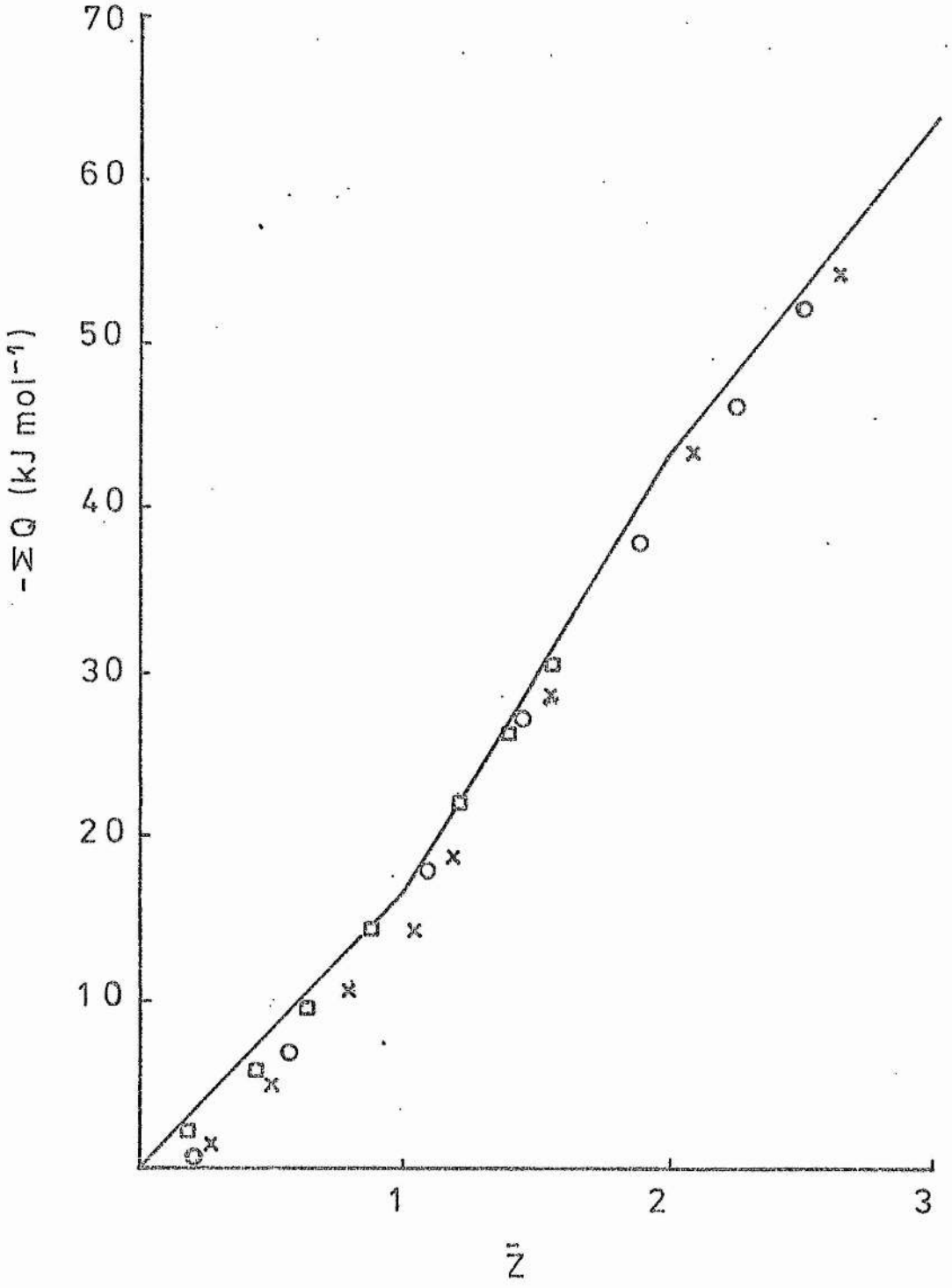

Figure 10 Enthalpic curve for the Ni (II) - Asparaginate system.

Heats of formation for cobalt(II)-asparaginate complexes.

These were obtained by titrating sodium hydroxide (120.0mM in the first experiment and 125.0mM in the others) into cobalt(II)-asparaginate solutions. The initial volume was 100.0ml.

Table 14. Calorimetric results for the Co(II)-asparaginate system.

Initial [Co(II)] mM	4.696	7.049	9.383	9.383	2.350
Initial [asn] mM	16.43	23.90	18.06	9.948	8.088
Initial [H] mM	22.59	33.14	30.36	22.25	11.17
volume added (ml)	heat evolved (joules)				
4.0	(10.201)	(10.201)	(10.054)	(10.348)	1.913
5.0					1.932
6.0	(5.552)	(10.604)	(11.054)	(11.454)	2.301
7.0					1.162
8.0	4.998	(5.915)	(11.678)	(11.729)	
10.0	5.145	4.470	(5.873)	(7.068)	
12.0	4.818	4.659	4.024	4.395	
14.0	3.829	4.907	4.745	4.476	
16.0	3.184	4.996	4.447	4.392	
18.0	2.179	4.526	5.141	2.514	
20.0		3.752	4.889		
22.0		3.007	4.684		
24.0		3.176	3.411		

Processing the data in RWCALCRD and RWSOLV gave the following results.

$$\Delta H_1^o = -11.95 \pm 0.50 \text{ kJ mol}^{-1}$$

$$\Delta H_2^o = -26.71 \pm 1.00 \text{ kJ mol}^{-1}$$

$$\triangle H_3^o = -36.40 \pm 1.50 \text{ kJ mol}^{-1}$$

s(29 readings) = 0.50

The enthalpic curve is shown in figure 11.

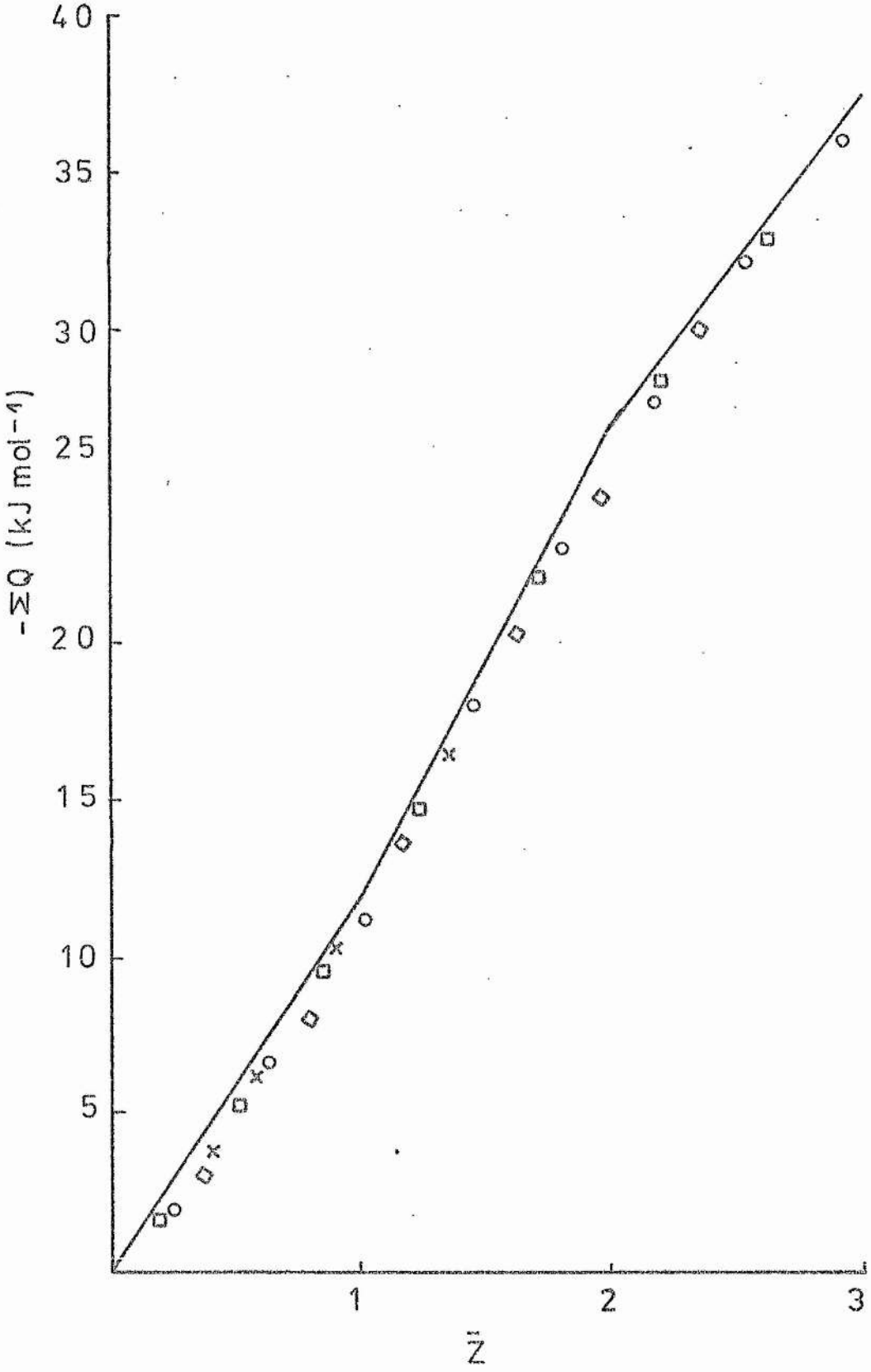

Figure 11 Enthalpic curve for the Co(II)-Asparaginate system.

Heats of formation for iron(II)-asparaginate complexes.

Values of ΔH^{o} for this system were not obtained because of the oxidative sensitivity of the system. The calorimeter described is not designed to incorporate an inert atmosphere, hence it was not possible to maintain anoxic conditions overnight. However the Fe(II)-amino-acid systems are considered sufficiently important to warrant closer investigation of the experimental difficulties of obtaining values of ΔH^{o}, with a view to modifying the existing calorimeter or constructing a new one. Failing that, it should be possible to obtain ΔH^{o} values by one of the following methods :-

(a) The van't Hoff isochore method (see Chapter 2), which has been successfully used by Raju and Mathur in determining ΔH^{o} for metal complexes of serinate and threoninate[142]. The applicability of this method depends on ΔH^{o} being constant over a range of temperatures (usually 10-40^{o}C).

(b) The alternative calorimetric method of using sealed ampoules containing the metal ion solution. The ligand solution is already positioned in the calorimeter, and the ampoule is then introduced and broken, usually by the stirrer. This method has been used by Pettit et al in their metal amino-acid studies[143].

Heats of formation for manganese(II)-asparaginate complexes.

These were measured by titrating sodium hydroxide (125.0 mM) into manganese(II)-asparaginate solutions. Precipitation occurred towards

the end of the titrations.

Table 15. Calorimetric results for the Mn(II)-asparaginate system.

Initial [Mn(II)] mM	5.027	7.545	10.04	10.04
Initial [asn] mM	9.794	14.32	22.38	28.12
Initial [H] mM	20.53	20.39	33.78	39.52
volume added (ml)		heat evolved (joules)		
5.0				
6.0	(12.055)	2.451		
7.0		1.212		
8.0	(11.933)	1.479	(5.762)	(4.590)
9.0		1.390	(4.891)	0.772
10.0	7.796	1.247	0.884	0.780
11.0		1.154	1.102	0.787
12.0	1.694	1.430	1.113	0.794
13.0			1.122	0.855
14.0	2.966			

Processing the data by RWCALCRD and RWSOLV gave the following values :

$$\Delta H_1^o = -\ 7.26 \pm 0.75 \text{ kJ mol}$$

$$\Delta H_2^o = -14.24 \pm 1.50 \text{ kJ mol}$$

$$s(21 \text{ readings}) = 0.75$$

The enthalpic curve is shown in figure 12.

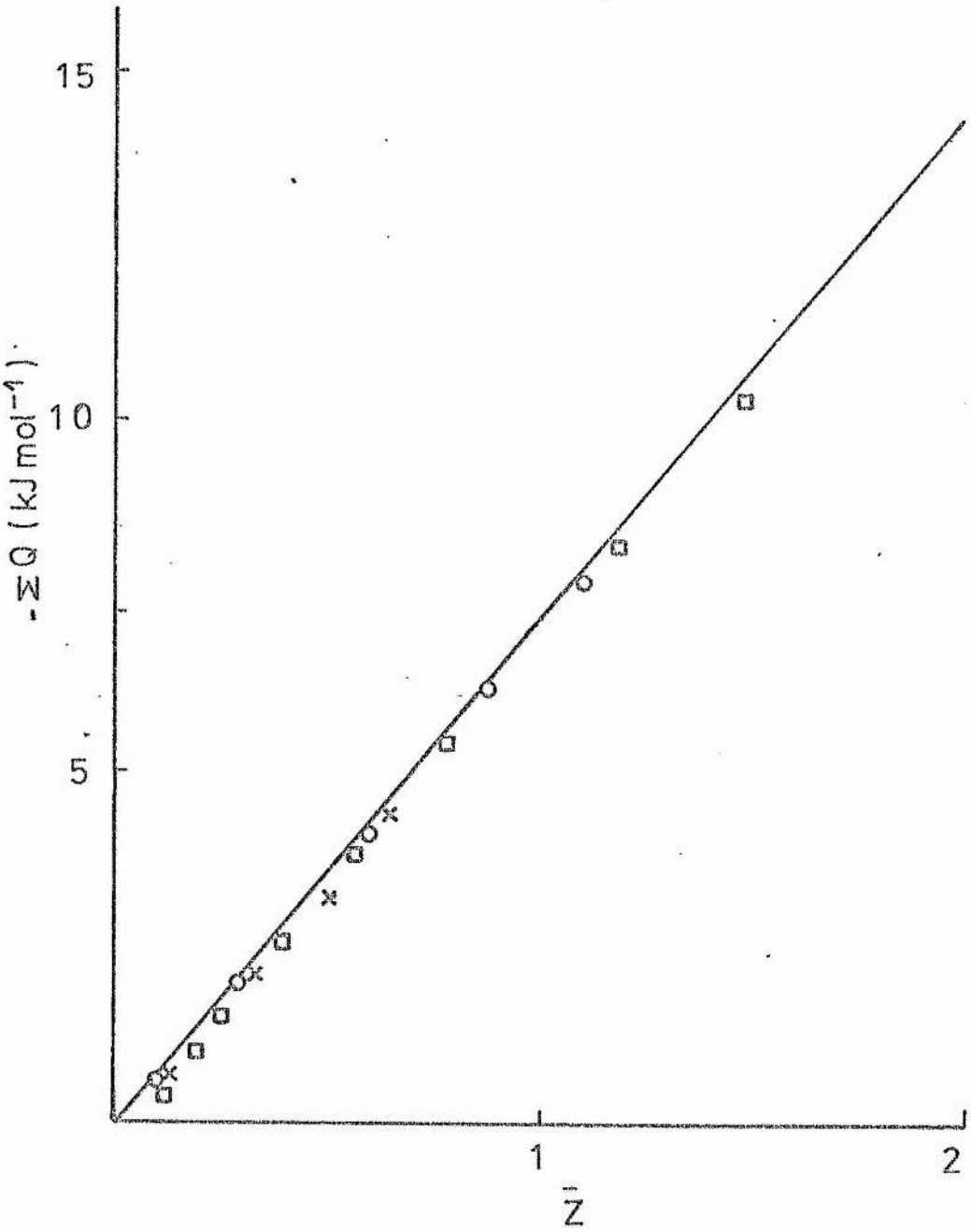

Figure 12. Enthalpic curve for the Mn(II)-Asparaginate system.

The homologue "paradox"

When Tanford and Shore[30] were measuring formation constants for cobalt(II)-amino-acid complexes, they found that the protonation constant for the amine site of glycinate was lower than the corresponding constant for the next higher homologue alaninate ($\log \beta_{101} = 9.69$ and 9.78 respectively), but that the formation constant for $Co(II).gly^{+}$ was higher than for $Co(II).ala^{+}$ ($\log \beta_{110} = 4.65$ and 4.27 respectively). They could offer no explanation for this observation.

The same phenomenon has been reported by Williams for asparaginate and glutaminate[29], as illustrated in the following table.

Table 16. Protonation constants and formation constants for metal ion complexes with glutaminate(gln) and asparaginate(asn).

Metal ion	$\log \beta$ value for gln complex	$\log \beta$ value for asn complex	Difference between $\log \beta$ values
H	9.640	9.303	+0.337
Mn(II)	2.863	3.102	-0.239
Fe(II)	4.432	4.366	+0.066
Co(II)	4.518	4.903	-0.385
Ni(II)	5.561	6.152	-0.591
Cu(II)	9.052	8.677	+0.375
Zn(II)	4.826	5.070	-0.244

From Table 16 it can be seen that whereas asparaginate has a lower protonation constant than glutaminate, the 1:1 complexes of asparaginate with Mn(II), Co(II), Ni(II) and Zn(II) have higher formation constants than for glutaminate with the same metal ions. A further anomaly exists with

the Cu(II), and to a lesser extent the Fe(II), complexes, where the constants for asparaginate are smaller than for glutaminate.

It was decided to investigate this phenonenon by calorimetry, and accordingly measurements of ΔH^{o} for complexes between glutaminate and nickel(II) and glutaminate and copper(II) were made.

Heats of protonation for glutaminate.

These were measured by titrating sodium hydroxide into acid solutions of the amino-acid. The initial volume was 100.0ml and the concentration of the titrant alkali was 449.0 mM in the first titration and 150.0mM in the others.

Table 17. Calorimetric results for the protonation of glutaminate.

Initial [gln] mM	39.10	24.62	24.62	23.09
Initial [H] mM	39.10	38.04	38.04	39.18
volume added (ml)		heat evolved (joules)		
2.0			13.314	13.122
4.0	5.199		13.438	11.525
6.0	3.751		12.405	12.505
8.0	1.275		13.677	2.346
10.0	4.262	12.577	9.719	2.079
12.0	0.598	1.430	1.165	2.330
14.0	2.912	1.833	1.294	2.481
16.0	0.220	1.427	1.647	2.361
18.0		1.175	1.565	2.347
20.0		1.308	0.966	2.445
22.0		1.388	1.272	

The data were processed in RWCALCRD and RWSOLV and the results were as follows :

$$\Delta H_1^o = -50.86 \pm 0.50 \text{ kJ mol}^{-1}$$

$$\Delta H_2^o = -55.28 \pm 1.00 \text{ kJ mol}^{-1}$$

s(35 readings) = 0.50

The enthalpic curve is shown in figure 13.

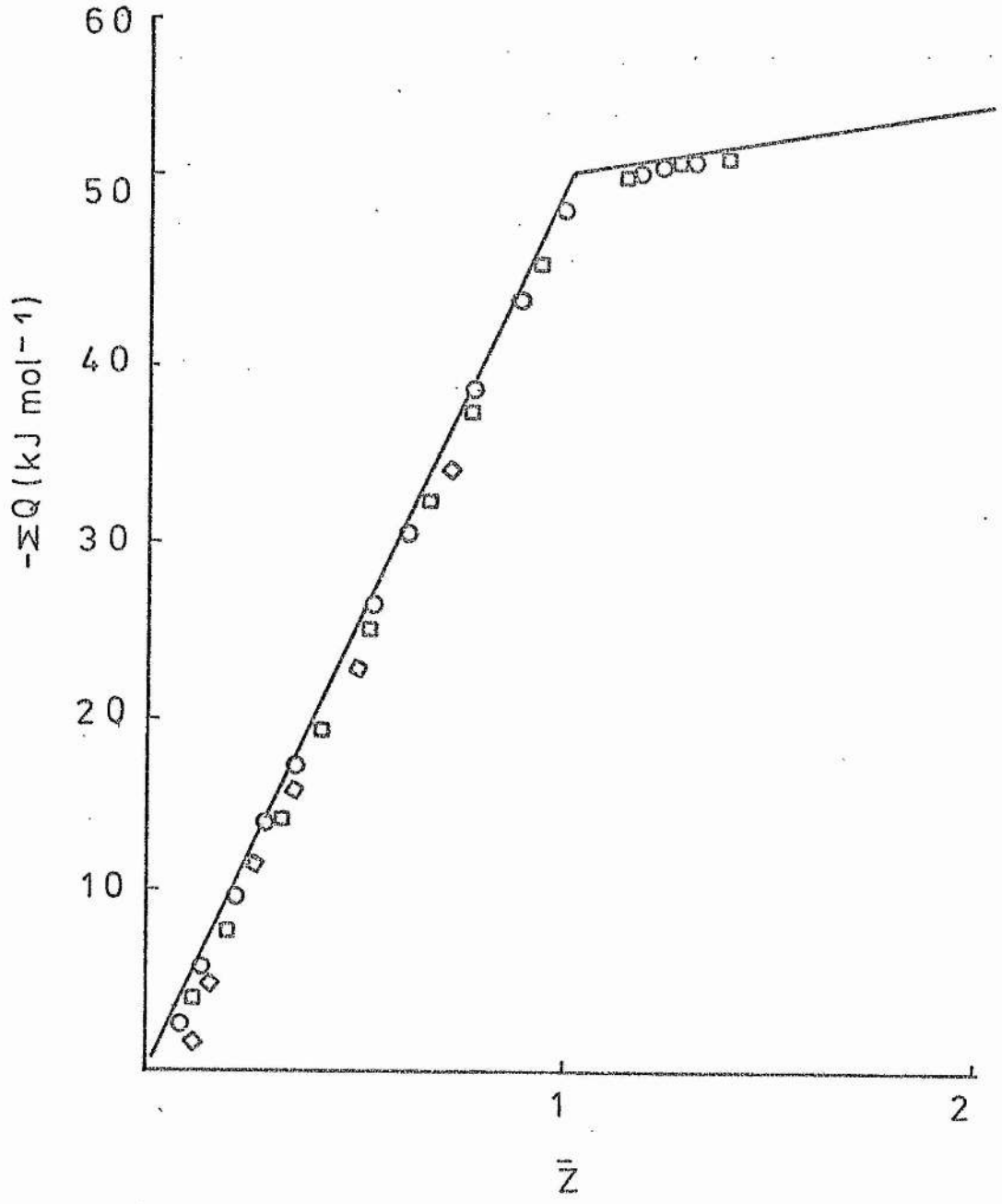

Figure 13 Enthalpic curve for Protonation of Glutaminate.

Heats of formation for nickel(II)-glutaminate complexes.

These were obtained by titrating sodium hydroxide (125.0mM) into Ni(II)-glutaminate solutions. The initial volume was 100.0ml.

Table 18. Calorimetric results for the Ni(II)-glutaminate system.

Initial [Ni(II)] mM	9.682	9.682	7.274
Initial [gln] mM	18.54	27.39	23.26
Initial [H] mM	24.58	33.43	27.80
volume added (ml)		heat evolved (joules)	
2.0	(8.748)	(9.325)	(10.671)
4.0	5.198	(6.670)	(8.631)
6.0	5.152		6.252
7.0		8.889	
8.0	5.253	2.499	5.966
10.0	5.613	5.509	5.769
12.0	5.559	5.612	5.877
14.0	5.570	5.608	6.201
16.0	5.325	5.984	6.478
18.0		6.258	6.091
20.0		6.709	5.344
22.0		6.245	2.949
24.0		4.940	

The data were processed by RWCALCRD and RWSOLV and the heats of formation were :

$$\Delta H_1^o = -13.28 \pm 0.60 \text{ kJ mol}^{-1}$$

$$\Delta H_2^o = -36.09 \pm 1.20 \text{ kJ mol}^{-1}$$

$$\Delta H_3^o = -54.75 \pm 1.80 \text{ kJ mol}^{-1}$$

s(24 readings) = 0.60

The enthalpic curve is shown in figure 14.

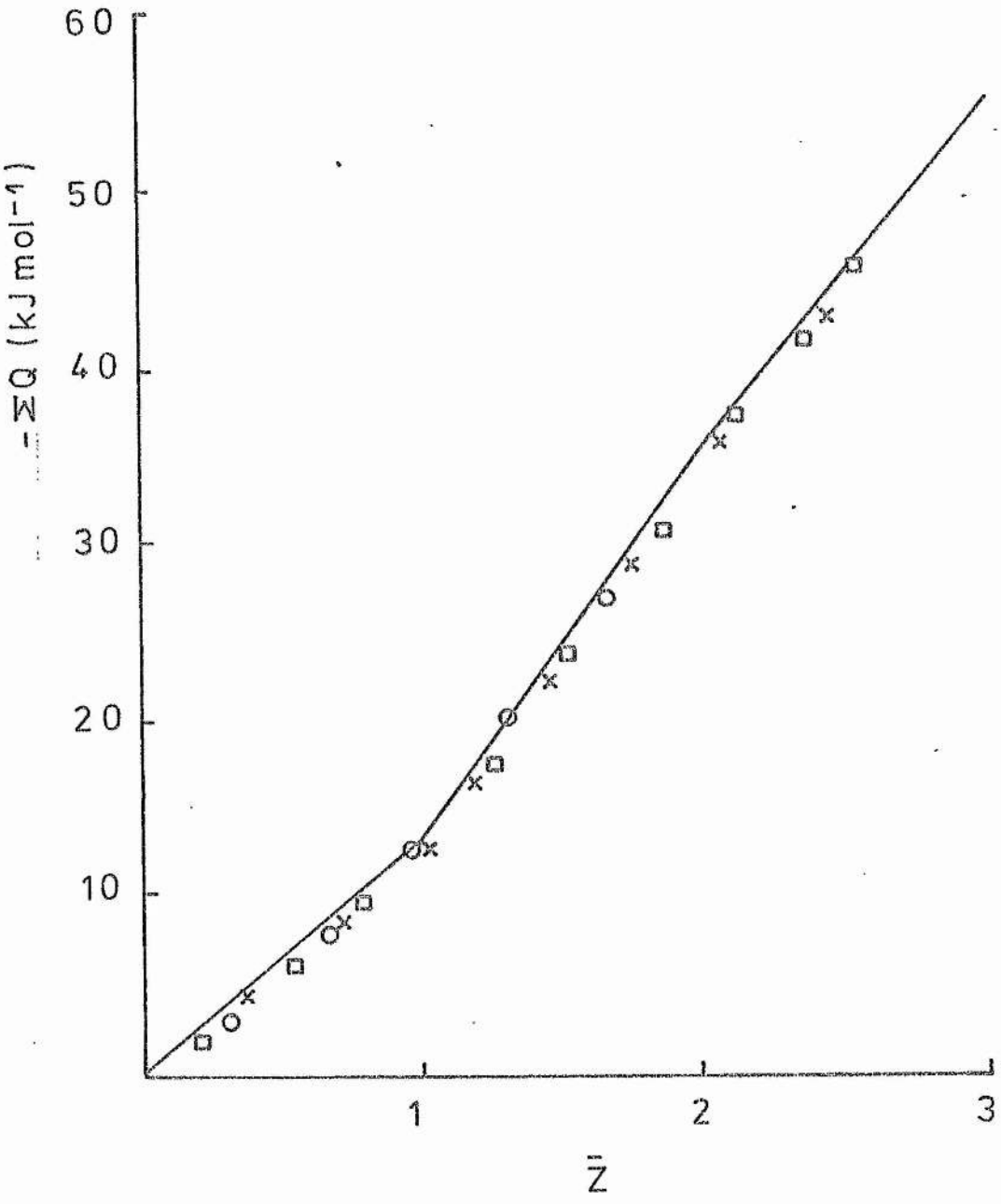

Figure 14. Enthalpic curve for the Ni (II) - glutaminate system.

Heats of formation for copper(II)-glutaminate complexes.

These were obtained by titrating sodium hydroxide (125.0mM) into solutions of Cu(II)-glutaminate. The initial volume was 100.0ml.

Table 19. Calorimetric results for the Cu(II)-glutaminate system.

Initial [Cu(II)] mM	7.139	9.503	9.503
Initial [gln] mM	15.17	19.26	19.65
Initial [H] mM	21.19	27.28	27.66
volume added (ml)		heat evolved (joules)	
2.0	8.412	8.652	8.988
4.0	11.476	8.533	7.945
6.0	10.304	9.554	8.953
8.0	9.536	9.842	9.893
10.0	8.627	9.511	8.939
12.0	7.624	8.207	9.213
14.0	7.927	8.520	9.059
16.0	7.906	8.070	7.576
18.0	1.229	7.487	7.264
20.0		6.367	7.107
22.0		4.973	

Like the copper(II)-asparaginate system, the copper(II)-glutaminate system did not give meaningful constants when processed by RWCALCRD and RWSOLV.

The constants therefore had to be obtained graphically.

The results were : $\Delta H_1^o = -16.5 \pm 1.0$ kJ mol^{-1}

$\Delta H_2^o = -42.5 \pm 2.0$ kJ mol^{-1}

s(30 readings) = 1.0

The enthalpic curve is shown in figure 15.

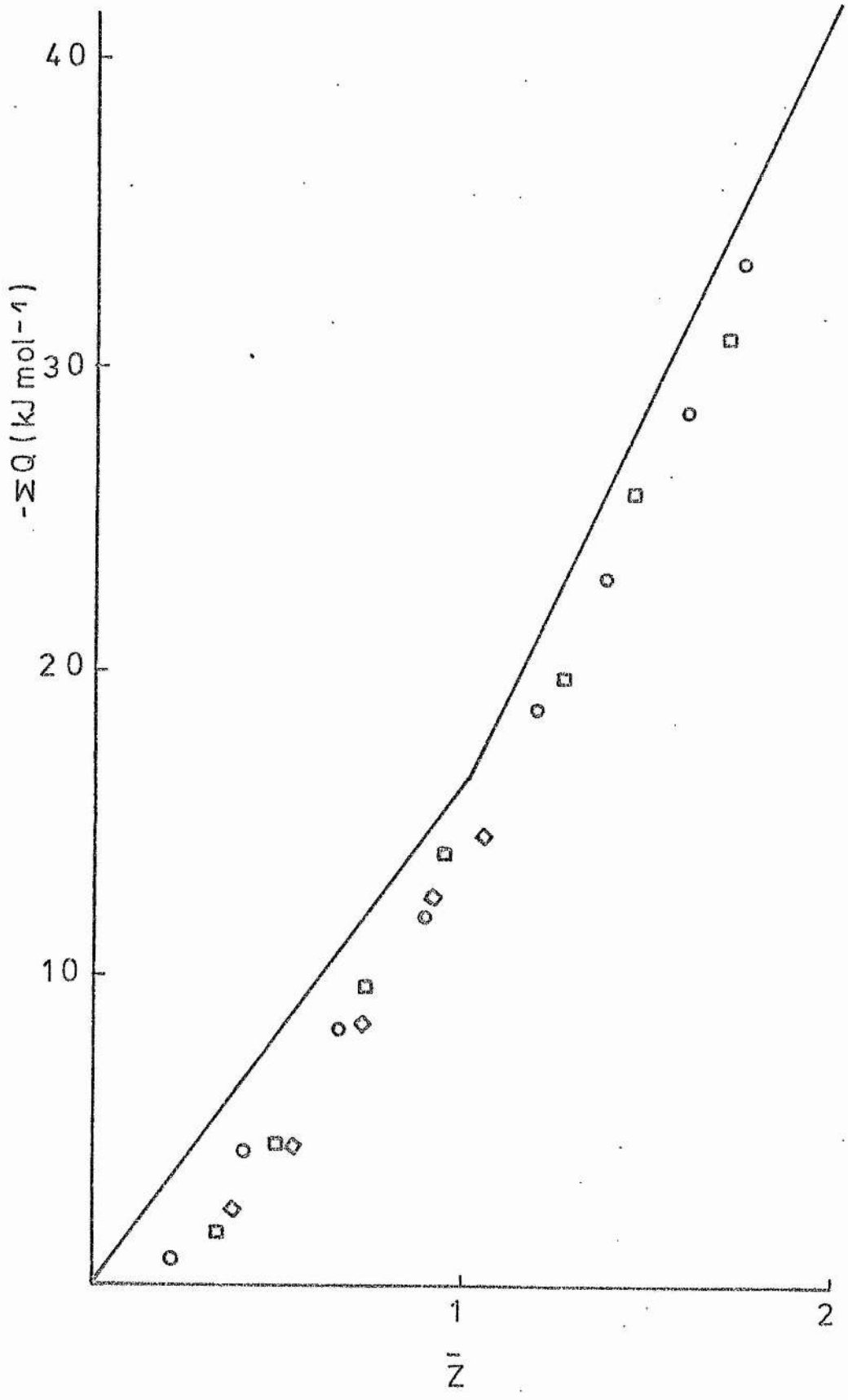

Figure 15 Enthalpic curve for the Cu (II) Glutaminate system

Comparison with other workers' results.

Although there has been a limited amount of potentiometric work done on metal asparaginate systems, there are very few calorimetric results for the same systems. The following are the literature values so far reported :-

$Cu(II).asn.^{+}$ $\triangle H^{o} = -26.3$ kJ mol^{-1} (Gergely et al[137])

$Cu(II).asn_2$ $\triangle H^{o} = -53.9$ kJ mol^{-1} (Gergely et al[137])

$\triangle H^{o} = -47.2$ kJ mol^{-1} (Barnes and Pettit[142])

CHAPTER 7. TERNARY COMPLEXES

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Chapter 7. TERNARY COMPLEXES

Ternary complexes in vivo and in vitro

It was mentioned in Chapter 1 that during Sarkar's work on copper transport he found low molecular weight ligands which could compete with albumin for the binding of copper(II)[14]. Further experiments revealed that these ligands were amino-acids, of which histidinate was the most important, followed by threoninate and glutaminate. The ternary complex Cu(II).his.thr. was identified during the course of this work[22], and was closely followed by the discovery of other ternary species, including those between albumin, copper(II) and histidinate or threoninate[23]. In the albumin ternary species, albumin binds to copper through the N-terminal aspartate residue[24].

A complex between copper, histidinate and glutaminate, the next higher homologue of asparaginate has also been discovered[25], and there is no reason to doubt that similar species with asparaginate will eventually be detected.

As part of their work on the involvement of biologically important ligands in ternary complexes, Sarkar et al made an in vitro potentiometric investigation of the formation of complexes between Cu(II), histidinate, and glutaminate or serinate[144]. They found that the ternary species had higher stability constants than the parent binary species BA_2. This phenomenon has been noted by several research groups in the last 10-15 years. Martin and Paris[145] and Petit-Ramel and Paris[146] observed that BAA^1 was more

stable than either BA_2 or BA_2^1 (A and A^1 were any two amino-acids and B was copper(II)), but offered no explanation for this phenomenon.

Perrin et al[97,147] observed that in their ternary complex studies formation of the ternary complexes was more favourable than the statistical case (see Chapter 8), and that this effect was more significant in Cu(II) complexes than Ni(II), primarily for steric reasons.

Gergely et al[148] have made an extensive survey of complex formation between copper(II) and two amino-acids. They found that although copper(II)-glycinate had a higher stability constant than any other copper-amino-acid complex, the presence of glycinate in a ternary complex destabilised the complex. Their explanation for this was that the bond angles were distorted and bond lengths increased in the glycine-containing ternary species.

In the present work, three ternary systems were examined. These were Cu(II).his.thr., and two systems so far undetected in vivo, Cu(II).asn.his. and Cu(II).asn.thr. The techniques used to study these systems were those which were employed in the previous binary complex study, namely potentiometry and calorimetry, in 3.00M(Na)ClO_4 at 25.0°C.

As far as computational aspects were concerned, SCOGS or MINIQUAD could be used to refine the formation constants obtained from the potentiometric measurements, but RWCALCRD in its existing form could not handle ternary species, so it was decided to calculate the heats of formation by hand. The method of calculation will be described later in

this chapter.

Before the study of the ternary systems was begun, some preliminary work on threoninate protonation and complexation with copper(II) in 3.00M(Na)ClO_4 at 25°C had to be carried out, as there were no literature values of formation constants for these species under these conditions.

Protonation constants for the threoninate ion.

Each protonation was studied independently, and in order to achieve this, ligand solutions without any added mineral acid or alkali were titrated with perchloric acid or sodium hydroxide to produce the formation curve which was constructed using RWZPLOT. The formation curve was independent of the ligand concentration, hence polynuclear species were assumed to be absent.

The analysis of the data using SCOGS gave the following constants :

$$\log \beta_{101} = 9.348 \pm 0.006$$

$$\log \beta_{102} = 11.925 \pm 0.008$$

s(115 readings) in titre = 0.251

Table 20 Protonation constants for the threoninate anion

Titration number	Ligand concentration mM	Mineral acid concentration in titrant mM	Initial volume (ml)	E^o (mV)
1	24.75	-39.90	24.97	411.4
2	24.75	39.67	24.97	411.6
3	34.63	-39.90	24.97	411.1
4	34.63	39.67	24.97	411.6
5	11.43	75.15	24.97	411.9

Negative acid concentrations in column 3 refer to alkali.

Titration No. 1

vol. added (ml.)	e.m.f. (mV)
0.00	28.3
0.10	-18.4
0.20	-34.7
0.30	-45.0
0.40	-52.7
0.50	-58.1
0.70	-67.0
1.00	-76.6
1.50	-87.9
2.00	-96.0
2.50	-102.7
3.00	-108.3
3.50	-113.2
4.00	-117.6
5.00	-125.4
6.00	-132.5

Titration No. 1 contd.

vol. added (ml.)	e.m.f. (mV)
7.00	-139.1
8.00	-145.5
9.00	-151.8
10.00	-158.5

Titration No. 2

vol. added (ml.)	e.m.f. (mV)
0.00	28.9
0.10	107.5
0.20	136.3
0.30	149.8
0.40	158.8
0.50	165.5
0.70	175.4
1.00	185.7
1.50	197.2
2.00	205.5

Titration No. 2 contd.

vol. added (ml.)	e.m.f. (mV)
2.50	211.9
3.00	217.4
3.50	222.0
4.00	226.5
5.00	233.7
6.00	239.8
7.00	245.3
8.00	250.2
9.00	254.7
10.00	258.9
11.00	262.6
12.00	266.1
13.00	269.6
14.00	272.7

Titration No. 3		Titration No. 4		Titration No. 5 contd.	
vol. added (ml.)	e.m.f. (mV)	vol. added (ml.)	e.m.f. (mV)	vol. added (ml.)	e.m.f. (mV)
0.00	39.3	0.00	48.8	0.40	197.3
0.10	-6.4	0.10	117.7	0.50	203.8
0.20	-23.9	0.20	139.9	0.60	209.0
0.30	-34.4	0.30	152.2	0.80	217.5
0.40	-42.3	0.40	160.2	1.00	224.3
0.50	-47.9	0.50	166.5	1.20	229.9
0.70	-56.9	0.70	176.1	1.40	234.9
1.00	-66.4	1.00	186.1	1.60	239.1
1.50	-77.5	1.50	197.3	1.80	243.0
2.00	-85.4	2.00	205.3	2.00	246.6
2.50	-91.8	2.50	211.6	2.50	254.3
3.00	-97.1	3.00	216.9	3.00	260.9
3.50	-101.6	3.50	221.4	4.00	271.8
4.00	-105.7	4.00	225.4	5.00	280.2
5.00	-112.8	5.00	232.2		
6.00	-118.9	6.00	238.0		
7.00	-124.3	7.00	243.1		
8.00	-129.3	8.00	247.7		
9.00	-134.0	9.00	251.8		
10.00	-138.6	10.00	255.7		
11.00	-142.9	12.00	262.5		
12.00	-147.2	14.00	268.7		
13.00	-151.5	16.00	274.3		
14.00	-155.9	18.00	279.4		
15.00	-160.5	Titration No. 5			
16.00	-165.3	0.00	33.5		
17.00	-170.4	0.10	156.2		
18.00	-176.1	0.20	177.7		
19.00	-182.5	0.30	189.1		

Formation constants for copper(II)-threoninate complexes

These were measured by titrating sodium hydroxide into copper(II)-threoninate solutions.

Table 21 Potentiometric results for the Cu(II) threoninate system

Titration number	[Cu(II)] mM	[thr] mM	[mineral acid] mM	Titrant alkali concentration mM	Initial volume(ml)	E° (mV)
1	4.614	8.736	3.789	39.90	24.97	412.6
2	4.614	7.888	3.789	39.90	24.98	409.9
3	9.228	17.98	7.579	39.98	24.97	411.5
4	9.228	9.617	7.579	39.98	24.97	408.8
5	23.13	23.14	19.000	39.98	24.97	409.0
6	13.88	27.20	11.40	39.98	24.97	408.3

Titration No. 1		Titration No. 2 contd.		Titration No. 3 contd.	
vol. added (ml.)	e.m.f. (mV)	vol. added (ml.)	e.m.f. (mV)	vol. added (ml.)	e.m.f. (mV)
0.80	244.2	3.00	218.4	6.50	227.3
1.00	242.5	3.20	215.3	7.00	223.8
1.50	237.8	3.40	212.2	7.50	220.3
2.00	232.8	3.60	208.8	8.00	216.4
2.50	227.2	3.80	205.2	8.50	212.3
3.00	221.1	4.00	201.5	9.00	207.9
3.50	214.0	4.40	192.6	9.50	203.3
4.00	206.0	4.60	188.5	10.00	198.0
4.50	196.8	4.80	183.6	Titration No. 4	
5.00	186.0	5.00	178.3	0.00	266.7
5.50	173.4	5.20	172.6	0.50	264.2
6.00	158.8	5.40	166.6	1.00	261.3
6.50	141.4	5.60	160.1	1.50	258.4
7.00	120.8	5.80	153.1	2.00	255.3
Titration No. 2		6.00	145.6	2.50	252.1
0.00	250.6	6.20	137.3	3.00	248.8
0.20	249.1	6.40	128.2	3.50	245.3
0.40	247.4	6.60	118.1	4.00	241.7
0.60	245.6	Titration No. 3		4.50	237.9
0.80	243.8	0.00	260.9	5.00	233.9
1.00	241.9	0.50	258.8	5.50	229.5
1.20	239.9	1.00	256.5	6.00	225.0
1.40	237.8	2.00	251.9	6.50	219.8
1.60	235.8	3.00	247.1	7.00	214.3
1.80	233.6	3.50	244.6	7.50	208.2
2.00	231.3	4.00	242.0	8.00	201.4
2.20	228.9	4.50	239.3	8.50	193.6
2.40	226.4	5.00	236.5	9.00	184.5
2.60	223.9	5.50	233.5	9.50	173.6
2.80	221.2	6.00	230.5		

Titration No. 5		Titration No. 5 contd.		Titration No. 6 contd.	
vol. added (ml.)	e.m.f. (mV)	vol. added (ml.)	e.m.f. (mV)	vol. added (ml.)	e.m.f. (mV)
0.00	284.0	19.00	218.3	13.00	211.1
1.00	281.1	20.00	212.7	14.00	204.9
2.00	278.2	21.00	206.4	15.00	198.0
3.00	275.3	22.00	199.3	16.00	190.2
4.00	272.3	23.00	191.0	17.00	181.5
5.00	269.3	Titration No. 6		17.50	176.8
6.00	266.3	0.00	261.9	18.00	171.8
7.00	263.3	1.00	258.9	18.50	166.4
8.00	260.3	2.00	255.8	19.00	160.7
9.00	257.2	3.00	252.6	19.50	154.5
10.00	254.0	4.00	249.3	20.00	148.1
11.00	250.7	5.00	246.0	20.50	141.0
12.00	247.4	6.00	242.5	21.00	133.3
13.00	243.9	7.00	239.0	21.50	124.8
14.00	240.3	8.00	235.1	22.00	115.3
15.00	236.4	9.00	231.0	22.40	106.7
16.00	232.4	10.00	226.7	22.80	97.2
17.00	228.1	11.00	222.0	23.20	85.8
18.00	223.4	12.00	216.8		

The data were processed in SCOGS and as hydroxy complexes are always a possibility in a copper system a search for such species was conducted. However none could be found, which is in agreement with the work of Sharma[149], and the system could be defined by only two constants, which are as follows.

$$\log \beta_{110} = 8.597 \pm 0.006$$

$$\log \beta_{210} = 16.031 \pm 0.013$$

s(141 readings) in titre = 0.178

Other literature values for these systems

(a) Protonation of threoninate.

Table 22 Other workers' results for the protonation of threoninate

$\log \beta_{101}$	$\log \beta_{102}$	Temperature and method	Ionic background	Reference
9.348	11.925	25° gl	3.00M	This work
9.12		20° gl	→ 0	71
9.00		25° pol	0.06M	150
9.00		25° pol	0.06M	151
8.86	11.10	20° gl	1.00M	152
9.26	11.47	20° gl	0 corr	153
9.01	11.15	30° gl	0 corr	
9.26	11.58	15° gl	0.20M	142
9.03	11.35	25° gl	0.20M	
8.71	11.01	40° gl	0.20M	
8.96	11.18	25° gl	0.10M	154
8.98	11.15	25° gl	0.05M	148
8.954	11.163	25° gl	0.15M	143

gl = measurement by glass electrode potentiometry

pol = measurement by polarography

0 corr = measurement corrected to zero ionic background

→ 0 = measurement extrapolated to zero ionic background

(b) Copper - threoninate system

Table 23. Other workers' results for the complexation of Cu(II) with threoninate

$\log \beta_{110}$	$\log \beta_{210}$	Temperature and method	Ionic background	Reference
8.597	16.031	25° gl	3.00M	This work
	14.54	25° pol	0.06M	151
8.44	15.40	20° gl	$\longrightarrow$ 0	153
8.41	15.32	20° gl	$\longrightarrow$ 0	
8.03	14.77	25° gl	0.05M	148
8.20	14.94	15° gl	0.20M	142
8.06	14.70	25° gl	0.20M	
7.87	14.34	40° gl	0.20M	
7.55	14.01	37° gl	0.15M	146
8.010	14.585	25° gl	0.15M	143

Formation constants for copper(II)-asparaginate-threoninate complexes.

This was the first of the ternary systems to be studied, and also the simplest, in that there were only four binary metal ligand species also present in the system. The usual potentiometric procedure was adopted, sodium hydroxide being titrated into a solution of copper ions and the two ligands, which were usually (although not always) in equimolar quantities. A total of six titrations were performed, the last one being at constant ligand concentration.

Table 24. Potentiometric results for the Cu(II) asn. thr. system

Titration number	[Cu(II)] mM	[asn] mM	[thr] mM	Initial [mineral acid] mM	Initial volume (ml)	E^{o} (mV)
1	2.311	9.572	9.661	1.898	24.97	410.4
2	6.933	6.671	5.766	5.694	24.97	408.9
3	9.255	9.726	9.888	7.597	24.97	410.7
4	11.57	11.74	11.84	9.504	24.97	411.1
5	13.90	13.88	13.82	11.42	24.97	410.4

The concentration of the titrant alkali was 39.99 mM in titrations 1 and 2 and 39.94 mM in titrations 3-5.

The initial concentrations in titration No. 6 were as follows:

In vessel : [Cu(II)] = 5.767mM ; [thr] = 5.706mM ; [asn] = 5.860mM ; [mineral acid] = 4.736mM

In burette : [thr] = 5.713mM ; [asn] = 5.887mM ; [OH^-] = 50.04 mM

Initial volume was 24.98 ml and E^o was 415.2 mV.

Titration No. 1		Titration No. 1 contd.		Titration No. 2 contd.	
vol. added (ml.)	e.m.f. (mV)	vol. added (ml.)	e.m.f. (mV)	vol. added)ml.)	e.m.f. (mV)
0.00	228.6	4.40	1.1	7.50	185.3
0.40	224.7	4.50	-29.2	8.00	175.7
0.80	220.4	4.60	-43.8	8.20	171.5
1.20	215.8	4.70	-53.5	8.40	167.1
1.60	210.4	4.80	-60.7	8.60	162.5
2.00	204.2	4.90	-66.4	9.00	152.6
2.40	197.0	5.00	-71.2	9.20	147.2
2.80	188.4	Titration No. 2		9.40	141.5
3.00	183.4	0.00	258.7	9.60	134.7
3.20	177.3	0.40	256.4	9.80	129.0
3.40	170.7	0.80	253.9	10.00	122.0
3.50	166.9	1.00	252.6	10.20	115.6
3.60	162.6	2.00	245.8	10.40	107.3
3.70	157.8	3.00	238.4	10.60	97.5
3.80	152.3	4.00	230.0	10.80	86.0
3.90	145.6	5.00	220.2	11.00	71.5
4.00	137.2	5.50	214.5	11.10	61.8
4.10	126.2	6.00	208.5	11.20	50.8
4.20	108.2	6.50	201.6	11.30	35.6
4.30	69.4	7.00	194.0	11.40	15.6

Titration No. 2 contd.

vol. added (ml.)	e.m.f. (mV)
11.50	-4.4
11.60	-17.5
11.80	-35.6
12.00	-52.4
12.50	-123.7

Titration No. 3

vol. added (ml.)	e.m.f. (mV)
0.00	261.9
1.00	258.0
2.00	253.7
3.00	249.1
4.00	244.2
5.00	238.9
6.00	233.1
7.00	266.7
8.00	219.4
9.00	211.1
10.00	201.4
10.50	196.4
11.00	190.6
11.50	184.2
12.00	177.5
12.40	171.6
12.80	165.6
13.20	158.7
13.60	151.6
14.00	143.8
14.40	135.3
14.80	125.8
15.00	120.6

Titration No. 3 contd.

vol. added (ml.)	e.m.f. (mV)
15.20	115.0
15.40	108.9
15.60	102.3
15.80	95.0
16.00	86.8
16.20	76.7
16.40	64.9
16.60	49.5
16.80	27.7
17.00	-27.2

Titration No. 4

vol. added (ml.)	e.m.f. (mV)
0.00	266.2
1.00	262.7
2.00	259.0
3.00	255.1
4.00	251.1
5.00	246.9
6.00	242.4
7.00	237.6
8.00	232.5
9.00	226.8
10.00	220.5
11.00	213.5
12.00	205.5
13.00	196.4
14.00	186.1
14.50	180.4
15.00	174.2
15.50	167.6

Titration No. 4 contd.

vol. added (ml.)	e.m.f. (mV)
16.00	160.7
16.50	153.0
16.80	148.2
17.10	143.0
17.40	137.4
17.70	131.4
18.00	125.1
18.30	118.2
18.70	107.8
19.00	98.8
19.50	80.7
20.00	55.2
20.40	22.6
20.80	-68.9
21.60	-149.5
22.00	-162.0

Titration No. 5

vol. added (ml.)	e.m.f. (mV)
0.00	269.2
1.00	266.3
2.00	263.1
3.00	259.9
4.00	256.5
5.00	253.0
6.00	249.5
7.00	245.7
8.00	241.8
9.00	237.6
10.00	233.1
12.00	223.2

Titration No. 5 contd.		Titration No. 6		Titration No. 6 contd.	
vol. added (ml.)	e.m.f. (mV)	vol. added (ml.)	e.m.f. (mV)	vol. added (ml.)	e.m.f. (mV)
13.80	212.5	0.00	255.5	7.00	148.7
14.50	207.7	0.40	252.4	7.20	142.0
15.00	204.0	1.00	247.5	7.40	134.7
15.50	200.2	1.50	243.0	7.60	126.4
16.60	196.1	2.00	238.0	7.70	121.7
16.50	191.8	2.50	232.8	7.80	116.7
17.00	187.3	3.00	227.2	7.90	111.2
17.50	182.4	3.50	220.9	8.00	105.0
18.00	177.3	4.00	214.0	8.10	97.4
18.50	171.9	4.20	211.0	8.20	88.2
19.00	166.1	4.40	207.9	8.30	76.1
19.50	160.0	4.80	201.1	8.40	57.8
20.00	153.4	5.00	197.4	8.50	18.9
20.50	146.6	5.20	193.6	8.60	-51.4
21.00	138.9	5.40	189.6	8.70	-74.7
21.50	130.3	5.60	185.4	8.80	-87.6
22.00	120.6	5.80	180.9	8.90	-96.9
22.50	109.6	6.00	176.3	9.00	-104.1
23.00	96.5	6.20	171.3		
23.50	80.3	6.40	166.2		
24.00	58.3	6.60	160.7		
24.60	15.8	6.80	154.8		

The data was analysed using SCOGS and the following ternary species was discovered:

$$\text{Cu(II). asn.thr. , } \log \beta = 16.471 \pm 0.026$$

s(198 readings) in titre = 0.295

Formation constants for copper(II)-asparaginate-histidinate complexes

The Cu(II). asn.his. system was more complex, because of the existence of several protonated binary histidinate-copper species. There was therefore the possibility of formation of a protonated ternary species.

The binary histidinate constants used were those which had been obtained previously in this laboratory[26-27]. The formation constants of the ternary complexes were measured by titrating sodium hydroxide into solutions containing an approximately 1 : 1 : 1 molar ratio of Cu(II) : asn : his.

Table 25. Potentiometric results for the Cu(II). asn.his. system

Titration number	[Cu(II)] mM	[his] mM	[asn] mM	[Mineral acid] mM	titrant [OH^-] mM	Initial vol. (ml)	E^o (mV)
1	2.309	2.609	2.557	1.897	39.92	24.97	412.9
2	4.630	4.626	4.921	3.802	39.92	24.97	413.1
3	6.958	7.140	7.140	5.714	39.92	24.97	413.1
4	9.250	9.283	9.395	7.597	39.92	24.97	411.8
5	23.13	23.09	23.13	19.00	79.90	24.98	412.8
6	13.88	14.25	14.06	11.40	79.90	24.97	411.6
7	23.16	24.62	24.55	19.02	399.5	24.97	405.0
8	23.16	24.62	24.55	19.02	249.9	24.97	407.0

Titration No. 1		Titration No. 1 contd.		Titration No. 2 contd.	
vol. added (ml.)	e.m.f. (mV)	vol. added (ml.)	e.m.f. (mV)	vol. added (ml.)	e.m.f. (mV)
0.00	215.4	2.90	100.9	4.00	157.0
0.10	212.6	3.00	96.0	4.25	151.3
0.20	209.5	3.10	90.7	4.50	145.7
0.30	206.5	3.20	85.1	4.75	140.1
0.40	203.3	3.30	78.9	5.00	134.5
0.50	199.8	3.40	72.1	5.25	128.8
0.60	196.4	3.50	64.2	5.50	123.1
0.70	192.7	3.60	54.5	5.75	117.4
0.80	188.9	3.70	41.0	6.00	111.3
0.90	184.9	3.80	27.6	6.20	106.1
1.00	180.9	3.90	8.1	6.40	100.8
1.10	176.6	4.00	-18.3	6.60	95.3
1.20	172.5	Titration No. 2		6.80	89.3
1.30	168.4	0.00	229.5	7.00	82.9
1.40	164.0	0.25	227.0	7.20	75.7
1.50	159.9	0.50	223.9	7.30	71.6
1.60	155.7	0.75	220.5	7.40	67.7
1.70	151.6	1.00	217.0	7.50	63.3
1.80	147.5	1.25	213.1	7.60	58.4
1.90	143.3	1.50	209.1	7.80	48.0
2.00	139.4	1.75	204.8	8.00	34.7
2.10	135.2	2.00	200.3	8.20	17.1
2.20	131.1	2.25	195.4	8.40	-11.7
2.30	127.0	2.50	190.4	8.60	-103.6
2.40	123.0	2.75	185.0	Titration No. 3	
2.50	118.8	3.00	179.7	0.00	233.3
2.60	114.5	3.25	174.0	0.25	231.3
2.70	110.1	3.50	168.4	0.50	229.2
2.80	105.6	3.75	162.7	0.75	227.0

Titration No. 3 contd.

vol. added (ml.)	e.m.f. (mV)
1.00	224.7
1.40	220.8
1.70	217.7
2.00	214.4
2.40	209.7
2.80	204.7
3.20	199.4
3.60	193.7
4.00	187.8
4.40	181.5
4.80	175.1
5.20	168.6
5.60	162.1
5.80	158.8
6.10	153.9
6.40	149.0
6.70	143.9
7.00	139.3
7.30	134.5
7.60	129.8
7.90	125.0
8.20	120.1
8.50	115.0
8.80	109.8
9.10	104.5
9.40	98.9
9.70	92.9
10.00	86.5
10.30	80.2
10.60	72.0

Titration No. 3 contd.

vol. added (ml.)	e.m.f. (mV)
11.00	60.2
11.20	53.2
11.40	45.2
11.60	36.2
11.80	25.4
12.00	12.1
12.20	-7.2
12.40	-45.4

Titration No. 4

vol. added (ml.)	e.m.f. (mV)
0.00	240.0
0.50	237.1
1.00	233.8
1.50	230.3
2.00	226.6
2.50	222.6
3.00	218.4
3.50	213.8
4.00	208.8
4.50	203.6
5.00	198.1
5.50	192.2
6.00	186.1
6.50	179.8
7.00	173.3
7.50	166.7
8.00	160.2
8.50	153.6
9.00	147.2
9.50	140.8
10.00	134.2

Titration No. 4 contd.

vol. added (ml.)	e.m.f. (mV)
10.50	127.9
11.00	121.3
11.50	114.5
12.00	107.4
12.50	99.9
13.00	91.8
13.20	88.3
13.40	84.6
13.60	80.8
13.80	76.7
14.00	72.5
14.20	67.9
14.40	62.9
14.60	57.7
14.80	51.9
15.00	45.5
15.20	38.3
15.40	30.1
15.60	20.8
15.80	9.2
16.00	-6.8
16.20	-33.4

Titration No. 5

vol. added (ml.)	e.m.f. (mV)
0.00	254.3
1.00	249.6
2.00	244.5
2.50	241.8
3.00	238.9
3.50	235.9
4.00	232.7

Titration No. 5 contd.

vol. added (ml.)	e.m.f. (mV)
4.50	229.3
5.00	225.8
5.50	222.0
6.00	218.1
6.50	213.8
7.00	209.2
7.50	204.8
8.00	200.0
8.50	194.8
9.00	189.4
9.50	183.9
10.00	178.2
10.50	172.8
11.00	166.9
11.50	161.0
12.00	155.2
12.50	149.1
13.00	143.6
13.50	137.9
14.00	132.1
14.50	126.4
15.00	120.4
15.50	114.3
16.00	108.1
16.50	101.4
17.00	94.3
17.50	86.4
17.80	81.5
18.00	78.0

Titration No. 5 contd.

vol. added (ml.)	e.m.f. (mV)
18.20	74.3
18.40	70.4
18.60	66.2
18.80	61.6
19.00	56.8
19.20	51.6
19.40	45.8
19.60	39.6
19.80	32.4
20.00	24.3
20.20	15.5
20.40	4.2
20.60	-11.8
20.80	-37.5

Titration No. 6

vol. added (ml.)	e.m.f. (mV)
0.00	245.3
0.50	241.6
1.00	237.4
1.50	232.8
2.00	227.8
2.50	222.3
3.00	216.3
3.50	209.5
4.00	202.3
4.20	199.2
4.40	196.1
4.60	192.8
4.80	189.5
5.00	186.0

Titration No. 6 contd.

vol. added (ml.)	e.m.f. (mV)
5.20	182.5
5.40	179.0
5.60	175.4
5.80	171.8
6.00	167.6
6.20	164.6
6.40	160.9
6.60	157.3
7.00	150.1
7.20	146.4
7.40	142.9
7.60	139.3
7.80	135.7
8.00	132.1
8.20	128.5
8.40	124.7
8.60	121.1
8.80	117.3
9.00	113.5
9.20	109.5
9.40	105.4
9.60	101.3
9.80	96.9
10.00	92.2
10.20	87.6
10.40	82.3
10.60	76.8
10.80	70.7
11.00	64.0

Titration No. 6 contd.

vol. added (ml.)	e.m.f. (mV)
11.20	56.4
11.40	48.1
11.60	38.4
11.80	26.8
12.00	11.7
12.20	-10.1

Titration No. 7

vol. added (ml.)	e.m.f. (mV)
0.00	245.7
0.10	243.7
0.20	241.6
0.30	239.2
0.40	236.7
0.50	234.1
0.60	231.4
0.70	228.6
0.80	225.7
0.90	222.5
1.00	219.2
1.10	215.4
1.20	211.6
1.30	207.6
1.40	203.5
1.50	198.9
1.60	194.2
1.70	189.1
1.80	184.0
1.90	178.4
2.00	172.7
2.10	166.6

Titration No. 7 contd.

vol. added (ml.)	e.m.f. (mV)
2.20	160.5
2.30	154.6
2.40	148.4
2.50	142.3
2.60	136.4
2.70	130.1
2.80	124.1
2.90	117.9
3.00	111.8
3.10	105.2
3.20	98.3
3.30	91.1
3.40	83.0
3.50	74.6
3.60	64.7
3.70	53.5
3.80	39.7
3.90	21.2
4.00	-3.5

Titration No. 8

vol. added (ml.)	e.m.f. (mV)
0.00	244.8
0.20	242.8
0.40	240.1
0.60	237.1
0.80	234.1
1.00	230.9
1.20	227.3
1.40	223.5
1.60	219.5

Titration No. 8 contd.

vol. added (ml.)	e.m.f. (mV)
1.80	215.3
2.00	210.7
2.20	205.6
2.40	200.3
2.60	194.5
2.80	188.4
3.00	181.9
3.20	174.9
3.40	167.9
3.60	160.5
3.80	153.2
4.00	145.8
4.20	138.4
4.40	131.1
4.60	123.7
4.80	116.1
5.00	108.6
5.20	100.4
5.40	91.6
5.60	82.3
5.80	71.6
6.00	59.1
6.20	43.2
6.40	22.6
6.60	-8.7

The data was processed in SCOGS and two complexes were discovered, with the following formulae and formation constants.

$$Cu(II).asn.his \qquad \log \beta = 18.597 \pm 0.012$$

$$Cu(II).asn.his.H^{+}, \log \beta = 23.326 \pm 0.031$$

s(340 readings) in titre = 0.159

Although histidinate forms at least one hydroxy species with copper(II)[27,154-155], a search for complexes of formulae $Cu(II).asn.his.OH^{-}$ or $Cu(II).asn.his.(OH)_2^{2-}$ proved negative.

Formation constants for copper(II)-histidinate-threoninate complexes.

These were determined by titrating sodium hydroxide into solutions of copper(II) and the two ligands in approximately equimolar concentrations.

Table 26. Potentiometric results for the Cu(II).his.thr. system.

Titration number	[Cu(II)] mM	[his] mM	[thr] mM	[Mineral acid] mM	titrant [OH^-] mM	Initial vol. (ml)	E^o (mV)
1	2.318	2.380	2.394	1.904	39.92	24.97	411.2
2	4.626	4.805	4.705	3.799	40.00	24.98	411.5
3	9.250	9.438	9.583	7.597	40.00	24.98	412.6
4	15.72	15.58	15.58	12.91	70.10	24.97	413.3
5	23.13	23.14	23.14	19.00	70.10	24.98	412.5
6	23.16	24.58	24.43	19.02	399.5	24.97	404.4
7	23.16	24.58	24.43	19.02	249.9	24.97	404.4

Titration No. 1

vol. added (ml.)	e.m.f. (mV)
0.00	215.4
0.20	210.2
0.30	207.3
0.40	204.3
0.50	201.2
0.60	197.9
0.70	194.4
0.80	191.0
0.90	187.0
1.00	183.4
1.10	179.2
1.20	174.9
1.30	170.8
1.40	166.6
1.50	162.4
1.60	158.1
1.70	154.0
1.80	149.7
1.90	145.4
2.00	141.4
2.10	137.0
2.20	132.8
2.30	128.7
2.40	124.6
2.50	120.3
2.60	115.7
2.70	111.5
2.80	106.7
2.90	101.8

Titration No. 1 contd.

vol. added (ml.)	e.m.f. (mV)
3.00	95.8
3.10	90.1
3.20	84.5
3.30	77.1
3.40	69.7
3.50	61.9
3.60	51.7
3.70	39.7
3.80	24.8
3.90	3.3
4.00	-42.5

Titration No. 2

vol. added (ml.)	e.m.f. (mV)
0.00	226.5
0.20	224.0
0.40	221.3
0.60	218.5
0.80	215.5
1.00	212.3
1.20	208.9
1.40	205.3
1.60	201.8
1.80	197.9
2.00	193.9
2.20	189.7
2.40	185.4
2.60	180.9
2.80	176.4
3.00	171.5
3.20	167.0

Titration No. 2 contd.

vol. added (ml.)	e.m.f. (mV)
3.40	162.3
3.60	157.8
3.80	153.1
4.00	148.5
4.20	143.8
4.40	139.2
4.60	134.7
4.80	130.0
5.00	125.5
5.20	120.7
5.40	115.9
5.60	110.9
5.80	105.9
6.00	100.6
6.20	94.9
6.40	88.8
6.60	82.2
6.80	75.0
7.00	66.9
7.20	57.3
7.40	45.8
7.60	32.1
7.80	14.1
8.00	-15.2
8.20	-107.3

Titration No. 3

vol. added (ml.)	e.m.f. (mV)
0.00	237.7
0.50	234.5
1.00	231.3

Titration No. 3 contd.

vol. added (ml.)	e.m.f. (mV)
1.50	227.8
2.00	224.2
2.50	220.2
3.00	216.1
3.50	211.6
4.00	206.8
4.50	201.8
5.00	196.3
5.30	193.0
5.60	189.6
6.00	184.9
6.40	180.0
6.80	174.9
7.20	169.8
7.60	164.7
8.00	159.6
8.40	154.4
8.80	149.3
9.20	144.2
9.60	139.1
10.00	134.1
10.40	129.2
10.80	124.1
11.20	118.8
11.60	113.5
12.00	107.9
12.40	102.2
12.80	96.0
13.20	89.4

Titration No. 3 contd.

vol. added (ml.)	e.m.f. (mV)
13.60	82.3
14.00	74.7
14.40	65.7
14.80	55.5
15.20	43.1
15.60	27.4
16.00	4.9
16.40	-50.0

Titration No. 4

vol. added (ml.)	e.m.f. (mV)
0.00	248.5
0.40	246.2
0.80	243.7
1.20	241.1
1.60	238.4
2.00	235.6
2.40	232.6
2.80	229.5
3.20	226.2
3.60	222.7
4.00	219.0
4.40	215.0
4.80	210.8
5.20	206.4
5.60	201.8
6.00	196.9
6.40	191.7
6.80	186.3
7.20	180.7
7.60	175.1

Titration No. 4 contd.

vol. added (ml.)	e.m.f. (mV)
8.00	169.3
8.40	163.4
8.80	157.6
9.20	151.7
9.60	145.9
10.00	140.3
10.40	134.7
10.80	128.9
11.20	123.1
11.60	117.1
12.00	111.0
12.40	104.5
12.80	97.6
13.20	90.2
13.60	82.1
14.00	73.0
14.40	62.4
14.80	49.7
15.20	33.5
15.40	10.3
16.00	-39.1

Titration No. 5

vol. added (ml.)	e.m.f. (mV)
0.00	253.3
0.80	250.1
1.60	246.7
2.40	243.1
3.20	239.2
4.00	235.1
5.00	229.6

Titration No. 5 contd.

vol. Added (ml.)	e.m.f. (mV)
5.50	226.6
6.00	223.5
6.50	220.2
7.00	216.8
7.50	213.2
8.00	209.4
8.50	205.3
9.00	201.1
9.50	196.7
10.00	192.2
10.50	187.6
11.00	182.7
11.50	177.7
12.00	172.7
12.50	167.5
13.00	162.4
13.50	157.2
14.00	152.1
14.50	147.0
15.00	141.9
15.50	136.8
16.00	131.8
16.50	126.6
17.00	121.5
17.50	116.2
18.00	110.7
18.50	105.0
19.00	99.1
19.50	92.7

Titration No. 5 contd.

vol. added (ml.)	e.m.f. (mV)
20.00	85.9
20.50	78.3
21.00	70.3
21.50	60.9
22.00	49.8
22.50	35.9
23.00	17.7
23.50	-11.9
24.00	-110.9

Titration No. 6

vol. added (ml.)	e.m.f. (mV)
0.00	244.8
0.10	242.7
0.20	240.6
0.30	238.3
0.40	236.1
0.50	233.6
0.60	231.0
0.70	228.2
0.80	225.4
0.90	222.3
1.00	219.3
1.10	216.0
1.20	212.4
1.30	208.5
1.40	204.5
1.50	200.0
1.60	195.6
1.70	190.7
1.80	185.6

Titration No. 6 contd.

vol. added (ml.)	e.m.f. (mV)
1.90	180.3
2.00	174.8
2.10	169.1
2.20	163.1
2.30	157.2
2.40	151.0
2.50	145.0
2.60	138.9
2.70	132.9
2.80	126.8
2.90	120.8
3.00	114.9
3.10	108.5
3.20	101.9
3.30	95.0
3.40	87.5
3.50	79.5
3.60	70.3
3.70	60.0
3.80	47.9
3.90	32.3
4.00	13.1
4.10	-18.5

Titration No. 7

vol. added (ml.)	e.m.f. (mV)
0.00	244.2
0.20	241.7
0.40	238.9
0.60	236.1
0.80	233.0

Titration No. 7 contd.		Titration No. 7 contd.		Titration No. 7 contd.	
vol. added (ml.)	e.m.f. (mV)	vol. added (ml.)	e.m.f. (mV)	vol. added (ml.)	e.m.f. (mV)
1.00	229.7	3.00	181.1	5.00	107.5
1.20	226.2	3.20	174.2	5.20	99.2
1.40	222.4	3.40	167.0	5.40	90.6
1.60	218.5	3.60	159.7	5.60	81.2
1.80	214.2	3.80	152.3	5.80	70.3
2.00	209.7	4.00	144.8	6.00	57.3
2.20	204.7	4.20	137.5	6.20	41.3
2.40	199.4	4.40	130.1	6.40	19.3
2.60	193.7	4.60	122.7	6.60	-15.4
2.80	187.6	4.80	115.2		

After refinement in SCOGS, the following complexes and constants were obtained.

$$Cu(II).his.thr., \quad \log \beta = 18.613 \pm 0.016$$

$$Cu(II).his.thr.H^{+}., \quad \log \beta = 23.426 \pm 0.028$$

s(284 readings) in titre = 0.174

Although a complex of formula $Cu(II).his.thr.OH^{-}$ has been reported in the literature[154] it was not discovered in the present work.

Comparison with other workers' results.

As previously recorded in this chapter, a number of research groups have studied ternary complexation between a metal ion and two amino-acids. The copper(II).asn.thr. and copper(II).asn.his. systems have not yet received attention, but there are literature values for the copper(II).his.thr. system.

The formation constants obtained are listed below.

Cu(II).his.thr.,	$\log \beta = 17.464$	(Pettit et al[156])
	$\log \beta = 17.562$	(Freeman and Martin[154])
$Cu(II).his.thr.H^+$	$\log \beta = 21.43$	(Pettit et al)
	$\log \beta = 21.905$	(Freeman and Martin)
$Cu(II).his.thr.OH^-$,	$\log \beta = 7.00$	(Freeman and Martin)

Both sets of constants were obtained at 25°C and I = 0.1M.

Calorimetric measurements on the ternary species.

Before going ahead with the measurement of the heats of formation of the ternary complexes, it was necessary to obtain the heats of protonation for threoninate and the heats of formation for copper(II)-threoninate complexes. The instrumentation was as in previous calorimetric work in this laboratory.

Heats of protonation for threoninate.

These were measured by titrating sodium hydroxide (150.0mM) into solutions of ligand in perchloric acid. The initial volume was 100.0ml.

Table 27. Calorimetric results for the protonation of threoninate.

Initial [thr] mM	25.61	24.62	19.90
Initial [H] mM	40.11	39.12	30.79
volume added (ml)	heat evolved (joules)		
1.0	6.661	6.280	5.805
2.0	6.585	6.152	6.152
3.0	6.361	6.021	5.972
4.0	6.179	6.425	5.787
5.0	6.587	6.488	6.043
6.0	6.853	6.803	6.903
7.0	6.566	6.768	6.970
8.0	6.629	6.833	2.193
9.0	6.693	7.053	0.772
10.0	5.665	6.081	0.676
11.0	0.944	0.997	
12.0	1.006	1.324	1.959
14.0	2.265		1.995

Refinement of the data in RWCALCRD and RWSOLV gave the following heats of protonation:

$$\Delta H_1^{o} = -48.86 \pm 0.6 \text{ kJ mol}^{-1}$$

$$\Delta H_2^{o} = -63.89 \pm 1.0 \text{ kJ mol}^{-1}$$

s (37 readings) = 0.56

Heats of formation for copper(II)-threoninate complexes

These were measured by titrating sodium hydroxide (150.0mM) into Cu(II)-threoninate solutions. The initial volume was 100.0 ml.

Table 28. Calorimetric results for the Cu(II) threoninate system

Initial [Cu(II)] mM	4.632	2.321	9.274
Initial [thr] mM	10.45	4.882	19.94
Initial [H] mM	28.75	21.28	36.71
volume added (ml)	heat evolved (joules)		
2.0	12.161	10.575	13.170
4.0	12.408	10.299	11.917
6.0	13.455	14.506	12.655
8.0	13.157	14.993	13.259
10.0	12.941	13.876	12.681
12.0	11.278	12.178	12.707
14.0	11.755	10.893	11.863
16.0	11.694	4.063	11.200
18.0	10.672	1.676	11.510
20.0	4.491	0.398	10.347
22.0			11.045
24.0			4.881

The results, obtained graphically as for the other copper binary systems were as follows:

$$\Delta H^{o}_{1} = -18.0 \pm 1.5 \text{ kJ mol}^{-1}$$

$$\Delta H^{o}_{2} = -47.0 \pm 3.0 \text{ kJ mol}^{-1}$$

s(32 readings) = 1.50

Other literature values for these systems.

The protonation of the threoninate ion and the complex formation between it and Cu(II) ions has been examined by Izatt et al.[153] Their constants are extrapolated to zero ionic strength and are as follows:

Threoninate protonation $\Delta H_1^\circ = -41.8$ kJ mol^{-1} (c.f. -48.9 kJ mol^{-1})

$\Delta H_2^\circ = -47.7$ kJ mol^{-1} (c.f. -63.9 kJ mol^{-1})

Copper(II). threoninate $\Delta H_1^\circ = -22.2$ kJ mol^{-1} (c.f. -18.0 kJ mol^{-1})

$\Delta H_2^\circ = -47.7$ kJ mol^{-1} (c.f. -47.0 kJ mol^{-1})

Method of calculation of heats of formation for ternary complexes

The calculation of the heats of formation for the ternary complexes was much more complicated than for the binary complexes. The computer program RWCALCRD, plus RWSOLV, in its existing form was designed to take only binary species. Any attempt to generalise this program to include ternary species would have necessitated introducing a number of corrections for the formation of binary species, and since the extensive updating of a computer program is a time-consuming process, it was decided to calculate the ΔH° values by hand.

Initially the calorimetric titrations were simulated in HALTAFALL. The input consisted of the initial concentrations of the components of the system, and the formation constants for all the complexes which were known to exist in the system, and the printed output contained the added volume of titrant, the pH at each added volume, and the concentrations of all the complexes.

It was necessary to convert the concentrations of the complexes from

molar to number of moles present, which was accomplished using the formula:

$$\text{Number of moles} = \frac{\text{concentration (molar) X Total volume (ml)}}{1000}$$

and the change in the number of moles of each complex between two consecutive points could now be calculated.

The correction for the formation of water was constant for each equal step over the range of the titration (pH 3-7). To calculate this it was necessary to consider all protonated species which were known to be formed during the titration. A large contribution came from the neutralisation of mineral acid by alkali, and the remainder was accounted for by deprotonation of the ligand or of protonated metal :-ligand complexes. For the system Cu(II).asn.thr. the water correction involved just five terms, two protonated species for each ligand plus the change in pH of the solution, but in the histidinate systems there were no fewer than ten terms, including three protonated binary species, Cu(II).his.H^{2+}, Cu(II).his.$_2H^{+}$ and Cu(II).his.$_2H_2^{2+}$. The species Cu(II).his.OH was not included as insignificant amounts of it were formed during the course of a titration.

The water correction was expressed in terms of the change in number of moles of protons, according to an equation. For the Cu(II).his.thr. and Cu(II).asn.his. systems this equation was as follows :-

Total change in moles of protons =

$$= ph_{(2)} - ph_{(1)} + [AH]_{(1-2)} + 2[AH_2]_{(1-2)} + 3[AH_3]_{(1-2)} + [A^1H]_{(1-2)}$$
$$+ 2[A^1H_2]_{(1-2)} + [ABH]_{(1-2)} + [A_2BH]_{(1-2)} + 2[A_2BH_2]_{(1-2)} + [A^1BAH]_{(1-2)}$$

where (1-2) = (initial - final)

A = histidinate, A^1 = asparaginate or threoninate.

The sum of these terms was equivalent to the number of moles of water formed in going from No. 1 to point No. 2, and as ΔH^o_w was known, the required heat correction for the formation of water could be calculated for that point.

The calculation of the heats of formation for the ternary complexes was the most tedious in the whole method. In the Cu(II).asn.thr. system there were eight binary species formed during the titration and the amounts of these complexes formed at any point were known, so that the heat corrections for these species could be calculated. After applying these corrections to the experimental heat obtained in the titration, the contribution from the species Cu(II).asn.thr. could be calculated. As the amount of this complex formed at any stage of the titration was known, the required ΔH^o_f could be determined.

In the two histidinate systems there were 14 corrections to be taken into account and the resultant experimental heat was that due to formation of ABA^1 and A^1BAH (where A is histidinate and A^1 is the other ligand). The individual heats could then be extracted by a simple least squares process.

Heat of formation for the ternary complex Cu(II).asn.thr.

This was measured by titrating sodium hydroxide (39.99mM) into a solution of Cu(II) ions and the two ligands. The initial volume was 100.0ml.

Table 29. Calorimetric results for the Cu(II).asn.thr. system

Initial [Cu(II)] mM	2.311	6.933
Initial [asn] mM	9.661	5.766
Initial [thr] mM	9.572	6.671
Initial [H] mM	21.13	18.13
volume added (ml)	heat evolved (joules)	
1.6	2.729	
3.2	1.460	
4.0		5.885
6.4	4.569	
8.0	1.887	6.731
9.6	1.605	
12.0	3.812	6.512
14.0	2.319	
16.0	1.647	6.259
20.0		6.254
24.0		6.351
28.0		6.137
32.0		5.393

The resultant heat of formation for Cu(II).asn.thr. was -50.0 ± 2.5 kJ mol^{-1}

Heats of formation for Cu(II).asn.his. complexes

These were measured by titrating sodium hydroxide into a solution containing Cu(II) ions and the two ligands. The binary histidinate constants used were those obtained by Williams [26-27]. The initial volume was 100.0 ml, and the concentration of the titrant alkali was 39.92 mM in the first titration, 399.5 mM in the second and 249.9 mM in the third.

Table 30. Calorimetric results for the Cu(II).asn.his. system

Initial [Cu(II)] mM	6.958	23.16	23.16
Initial [asn] mM	7.140	24.55	24.55
Initial [his] mM	7.140	24.62	24.62
Initial [H] mM	19.99	68.19	68.19
volume added (ml)	heat evolved (joules)		
2.0		27.734	
3.6			28.315
4.0	4.806	30.406	
6.0		31.962	
6.4			28.662
8.0	5.915	30.291	
9.6			30.237
10.0		27.441	
11.2	4.257		
12.0		26.579	
12.8			29.815
14.0	8.400	26.207	
16.0	4.856		28.713
19.2			27.666
22.4			27.098
24.4	7.434		
28.0	5.164		

The following results were obtained.

Cu(II).asn.his., $\Delta H^{o} = -67.5 \pm 2.5$ kJ mol^{-1}

Cu(II).asn.his.H^{+}, $\Delta H^{o} = -88.2 \pm 5.0$ kJ mol^{-1}

Heats of formation for Cu(II).his.thr. complexes

These were measured by titrating sodium hydroxide into a solution of

Cu(II) ions and the two ligands. The initial volume was 100.0 ml, and the concentration of the titrant alkali was 40.00 mM for the first titration, 399.5 mM for the second, and 249.9 mM for the third.

Table 31. Calorimetric results for the Cu(II).his.thr. system

Initial [Cu(II)] mM	4.626	23.16	23.16
Initial [his] mM	4.805	24.58	24.58
Initial [thr] mM	4.705	24.43	24.43
Initial [H] mM	13.31	68.03	68.03
volume added (ml)	heat evolved (joules)		
2.0		27.109	
3.2			29.970
4.0	2.256	27.169	
6.0		31.412	
6.4			31.956
8.0	6.703	29.577	
9.6			31.014
10.0		27.908	
12.0	6.036	26.103	
12.8			31.576
14.0		16.878	
16.0	5.382		30.415
19.2			29.304
20.0	5.174		
22.4			28.316
24.0	5.469		
28.0	4.800		

The results obtained were as follows :-

$$Cu(II).his.thr., \quad \Delta H^{o} = -\ 67.4 \pm 3.0\ kJ\ mol^{-1}$$

$$Cu(II).his.thr.H^{+}., \Delta H^{o} = -103.5 \pm 6.0\ kJ\ mol^{-1}$$

Comparison with other workers' results.

Although formation constants have been obtained for the Cu(II)-histidinate-threoninate system, as yet there are no literature values of ΔH^{o} for this system.

Experimental thermogram.

This is a plot of total heat evolved against volume of titrant added. A thermogram of one of the Cu(II).asn.his. titrations is appended (figure 16), and superimposed upon it is a species distribution graph, with the volume axis being common to both graphs.

Species distribution graph.

The species distribution graph just mentioned can be generated from HALTAFALL. An equivalent graph, but with pH as the horizontal axis, can be produced using the St. Andrews version of COMICS, and it shows the proportion of a metal ion which is contained in a complex at any pH. A species distribution graph of the Cu(II).asn.his. system is included (figure 17), the concentrations used are those in blood plasma and are as follows : $[Cu(II)] = 1.37 \times 10^{-6}$ M, $[asn] = 4.39 \times 10^{-5}$M, $[his] = 8.15 \times 10^{-5}$M.

It can be seen from figure 17 that 30% of the Cu(II) is present in the ternary complex Cu(II).asn.his. at plasma pH (7.37-7.42), and is second

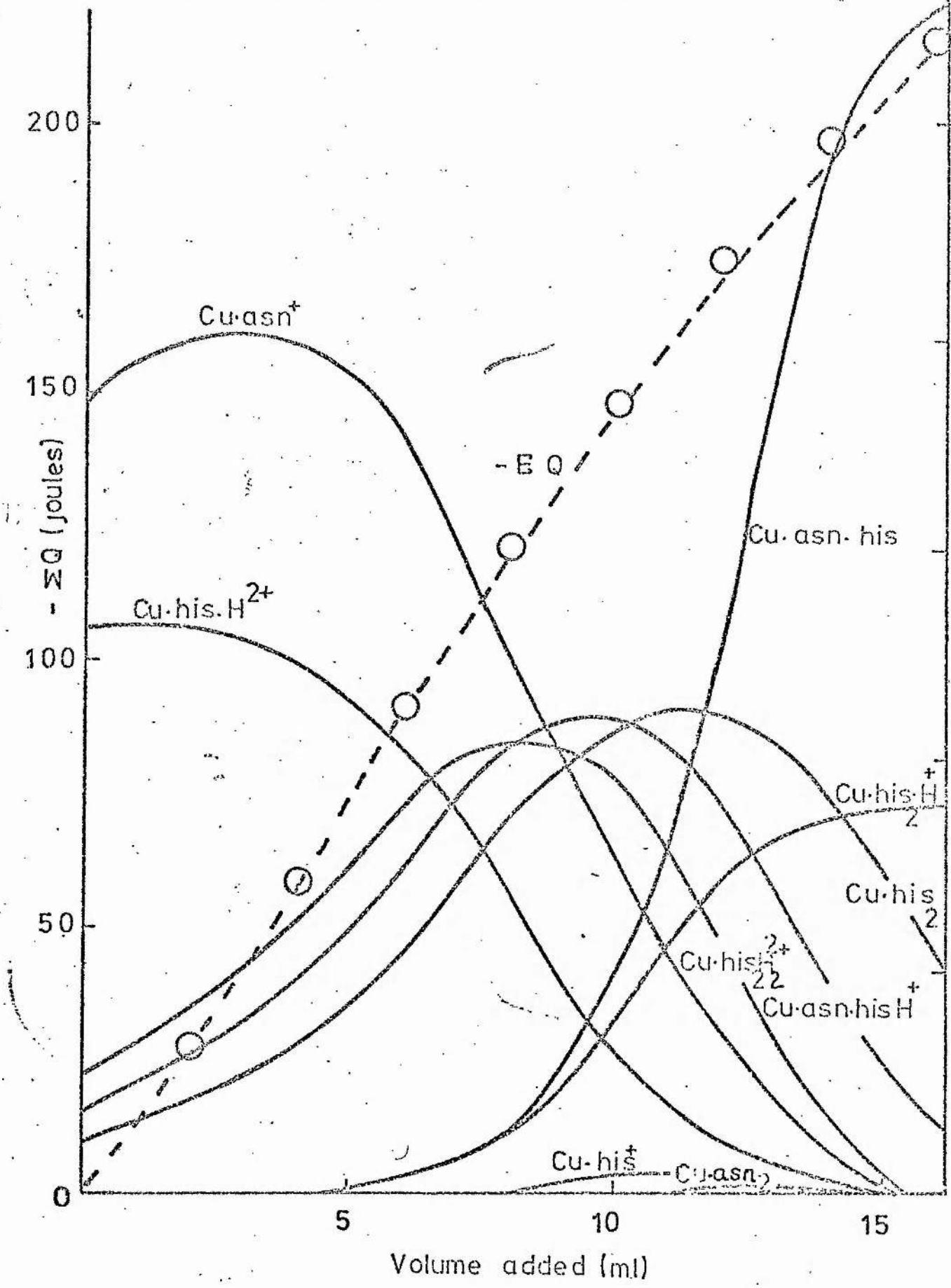

Figure 16. Experimental Thermogram for the Cu (II) - Asparaginate - Histidinate system.

only to Cu(II).his_2 at 55%. The protonated ternary species accounts for only 0.05% of the Cu(II). It is remarkable that such a high percentage of the Cu(II) appears in electrically uncharged form at plasma pH. Such neutral complexes have been correlated with membrane solubility[157], and thus it appears that binary bis and ternary 1:1:1 ligand complexes of copper are ideally suited for the role of copper-ion transport into cell membranes[158]. This observation is in agreement with the postulates of Sarkar and Kruck[159].

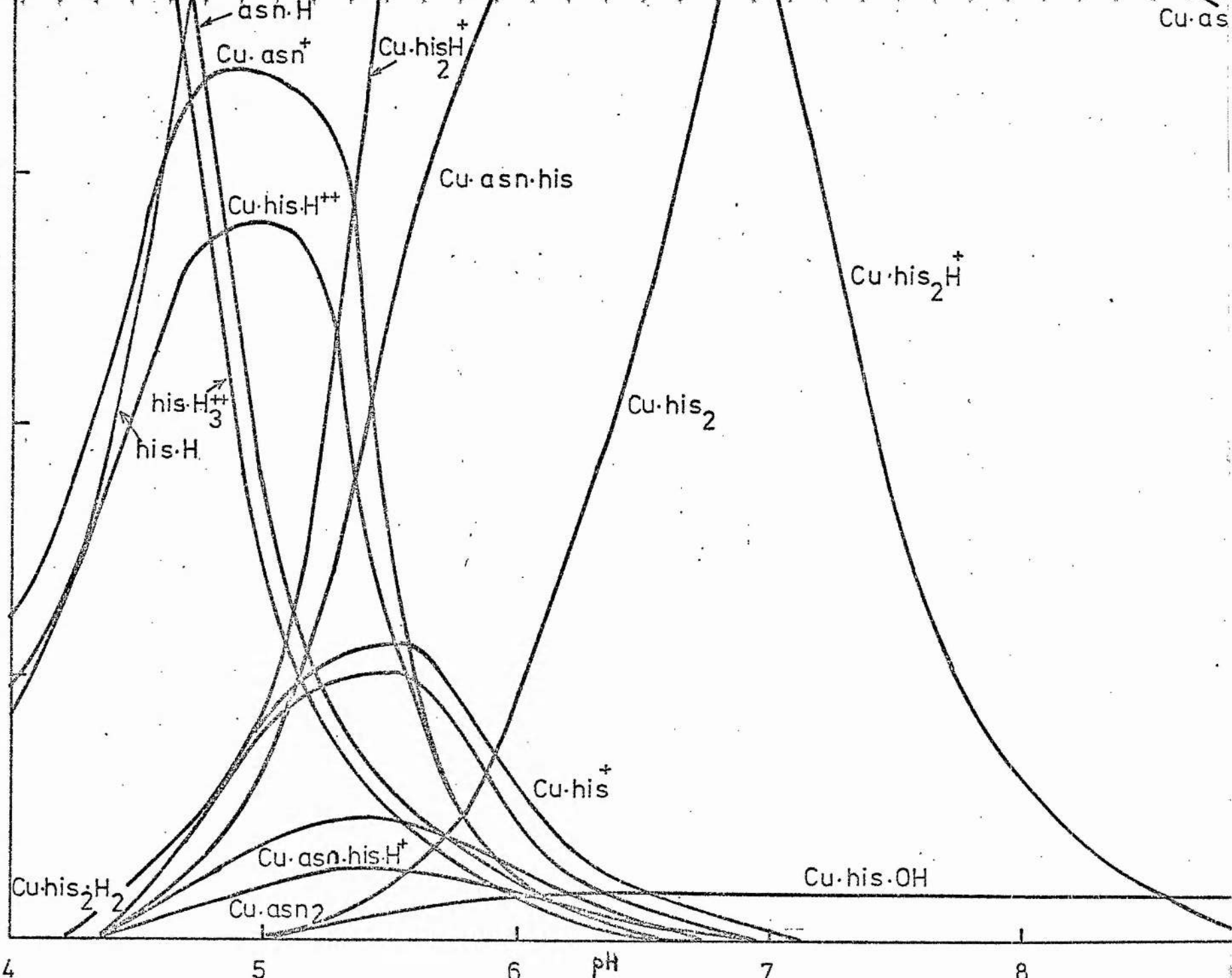

Figure 17. Species Distribution Graph for the Cu (II)–asn–his system.

CHAPTER 8. DISCUSSION

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Chapter 8. DISCUSSION

The results of this work can most effectively be discussed under several headings (a) the protonation of the asparaginate ion; (b) a review of thermodynamic work on metal asparaginate complexes; (c) the homologue "paradox", (d) the complexing reactions of the individual metal ions and (e) the ternary complexes.

(a) The protonation of the asparaginate ion.

In the last 25 years there have been ten determinations of the protonation constants for asparaginate, the results referring to a variety of temperatures and ionic strengths.

Figure 18, a plot of $\log \beta_{101}$ against the square root of the ionic strength I, shows the literature values, which appear to follow the lines dictated by the Guggenheim extension to the Debye-Huckel equation[162]

$$-\log f_z = 0.511 Z^+ Z^- \left(\frac{\sqrt{I}}{1+\sqrt{I}} - bI \right)$$

Where f is the activity coefficient, Z is the charge on the ion, and b is a constant which includes corrections for ionic strength variations of the dielectric constant of the medium and the effective sizes of the hydrated ions.

All these measurements were carried out by potentiometry using a glass/calomel electrode assembly, with two exceptions, one being the work of Azizov et al[160], where a mercury electrode was used in place of the glass electrode. As can be seen from figure 18 their value is the only one

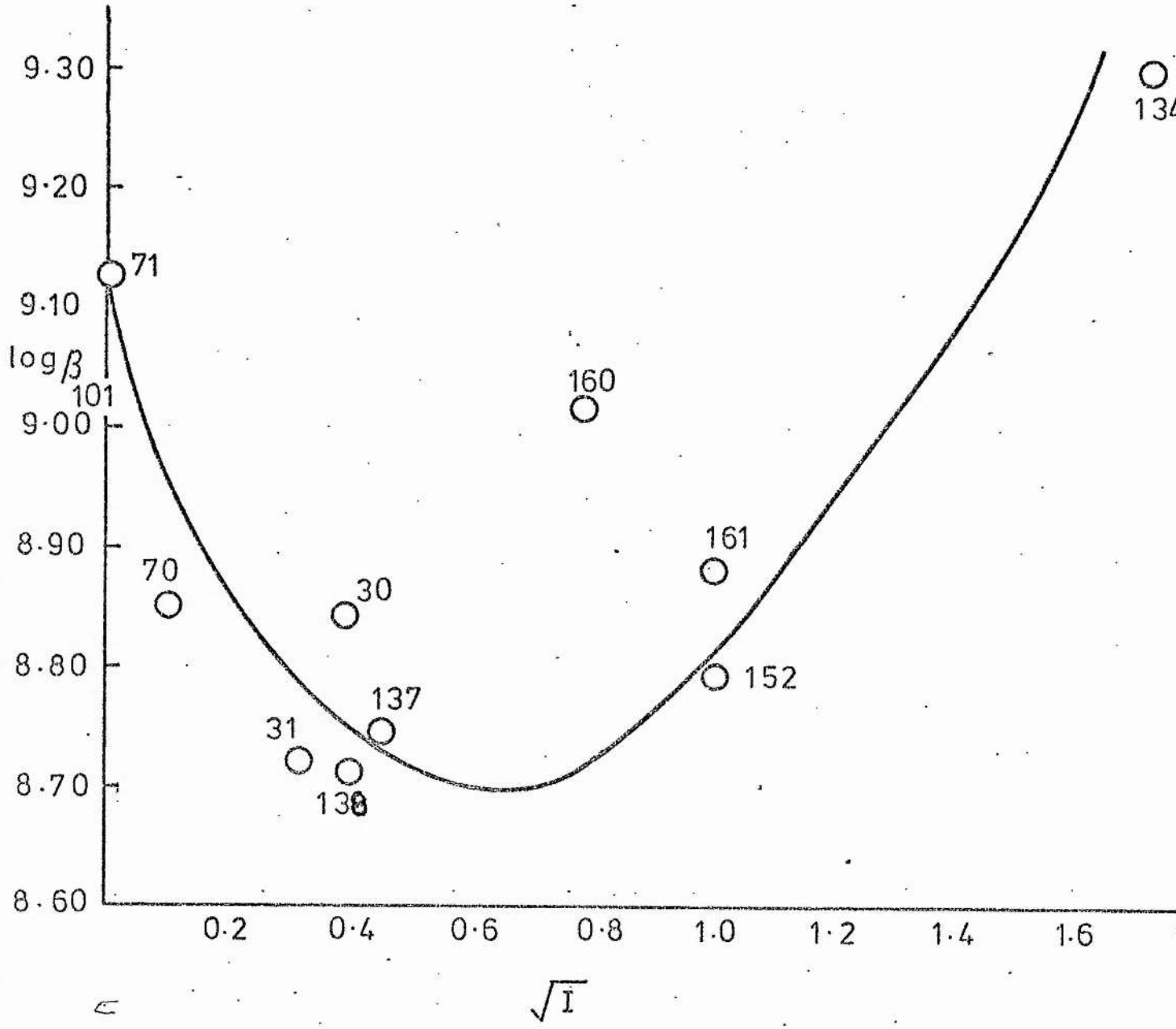


Figure 18 Graph of $\log \beta_{101}$ against $\sqrt{I}$ for the asparaginate ion.

which does not lie near the curve. The other exception was Rao and Subramanya[161], who determined log β_{101} by polarography.

Table 32. Thermodynamic parameters for protonation of asparaginate and glutaminate in 3.00M (Na)ClO_4 at 25°C

	asparaginate	glutaminate
$-\Delta G^{\circ}_{0,1}$	53.10	55.03
$-\Delta G^{\circ}_{1,2}$	14.76	15.55
$-\Delta G^{\circ}_{0,2}$	67.86	70.58
$-\Delta H^{\circ}_{0,1}$	50.50	50.86
$-\Delta H^{\circ}_{1,2}$	5.10	4.42
$-\Delta H^{\circ}_{0,2}$	55.60	55.28
$\Delta S^{\circ}_{0,1}$	8.9	14.0
$\Delta S^{\circ}_{1,2}$	32.4	37.4
$\Delta S^{\circ}_{0,2}$	41.3	51.4

ΔG° and ΔH° are expressed in units of kJ mol^{-1}, and ΔS° units are J $mol^{-1}K^{-1}$. The parameters $\Delta G^{\circ}_{0,1}$, $\Delta H^{\circ}_{0,1}$ and $\Delta S^{\circ}_{0,1}$ refer to the protonation of the amine site, the $\Delta_{1,2}$ parameters refer to the carboxylate site[29,134,163] and the $\Delta_{0,2}$ quantities are overall figures.

The effect of adding a $-CH_2$ group to asparaginate, making glutaminate, is reflected in glutaminate's slightly larger $-\Delta G^{\circ}$, $-\Delta H^{\circ}$ remaining essentially the same for both ligands. This increase in $-\Delta G^{\circ}$

arises from an approximately 20% increase in the entropy of protonating glutaminate as compared to asparaginate, which occurs because the glutaminate ligand is thought to possess more water of aquation which is shed upon protonation. A consequence of ΔH^{o} for glutaminate being roughly equal to ΔH^{o} for asparaginate is that ΔG^{o} and ΔS^{o} for glutaminate fit the $\Delta G^{o}_{-CO_2^-}$ versus $\Delta S^{o}_{-CO_2^-}$ plot[134] (figure 19).

For carboxylate protonation ΔG^{o} (or pK) is essentially entropy dependent[164]. Hansen et al suggested that $RCO_2^-H^+$ exists in solution as an ion pair and so ΔH^{o} approximates to zero and does not depend on the nature of R. Thus protonation is an electrostatic phenomenon and this is reflected in ΔS^{o} because the number of water molecules involved depend far more upon the charges of the ions concerned than upon the variety of groups being protonated. Izatt et al[165] produced evidence for such concepts in the form of linear plots of ΔG^{o} against ΔS^{o} at zero ionic strength. The slope of their plots was significantly close to that predicted by Bjerrum's theory of electrostatic interactions[166] (-243K compared to -218K). The data which have been compiled at 3.00M(Na)ClO_4 are fewer, but these parameters can also be said to lie near the line of slope -218K. Figure 19 shows the results compiled at 3.00M(Na)ClO_4. The solid line has slope -218K and the broken line has slope -243K.

The amine protonations may be contrasted with protonating carboxylate groups because (i) amine protonation is enthalpy dependent,

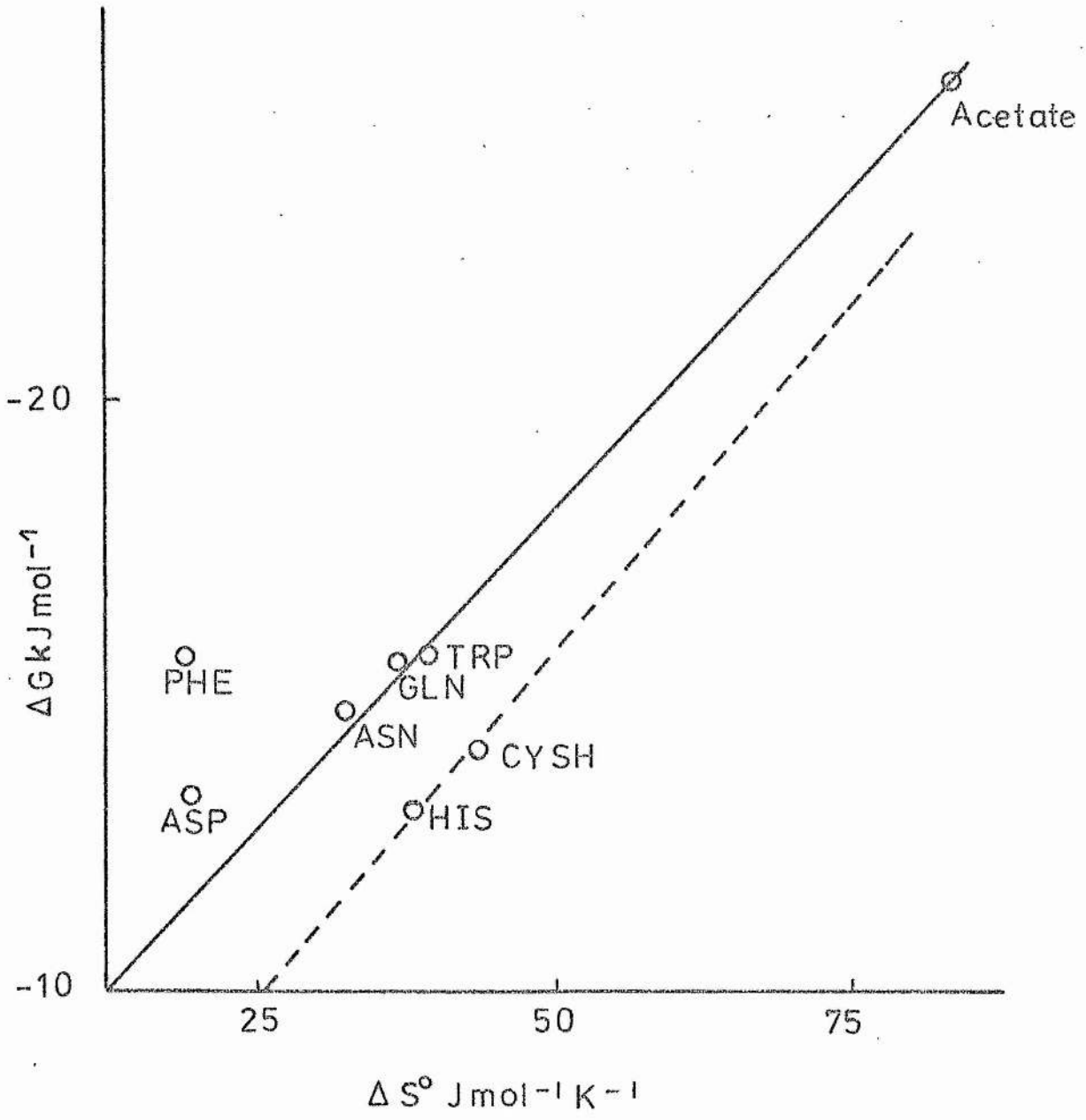


Figure 19. Plots of ΔG° v ΔS° for protonating carboxylate groups in perchlorate solutions.

(ii) substituents adjacent to the $-NH_2$ groups have more noticeable ΔH^o effects and (iii) whereas $-CO_2^-H^+$ interactions are charge-charge, $-NH_2H^+$ interactions are mainly charge-dipole and so electrostatic contributions to the entropies of protonation are frequently masked by large substituent effects.

(b) A review of thermodynamic work on metal asparaginate complexes.

In this section previous measurements of stability constants and heats of formation for metal asparaginate complexes will be discussed. The results of the present work will be tabulated and discussed in the light of previous work on these systems.

Up until 1950, very few quantitative investigations of complexation of amino-acids with metal ions had been made. Such neglect is not surprising because the nature of the equilibria concerned in such complex formation was not properly understood before the work by Bjerrum entitled "Metal Ammine Formation in Aqueous Solution"[167].

The first published formation constants for complexes between asparaginate and metal ions were by Albert[70], who calculated $\log \beta_{210}$ values for asparaginate complexes with Zn(II), Cu(II), Ni(II), Co(II), Fe(II)/(III) Mn(II), Cd(II) and Mg(II). For some reason, the $\log \beta_{110}$ values were not quoted, and, because he worked at a ratio of ligand to metal of 2:1, he did not obtain any tris complexes. Perkins[71], using the same approach, extended the work to Be(II) and Hg(II).

At about the same time as Perkins' work was published, Tanford

and Shore[30] undertook an investigation of the complexation of Co(II) with glycinate, alaninate, argininate and asparaginate, and obtained constants for AB^+, A_2B and A_3B^- complexes. Their results indicated that the presence of the amide group in asparaginate changed the structure of the chelating group.

All these previously discussed measurements were made using potentiometry, which has been the most popular method for measuring stability constants over the years, for reasons discussed in Chapter 1. The technique of ion-exchange does not feature prominently in stability constant measurements, but in 1954 Schubert[168] used ion-exchange to measure stability constants for complexes between various simple biologically important ligands and Ca(II), Sr(II), Ba(II) and Ra(II) at pH = 7.2. The constants for the asparaginate complexes were : Ca(II).asn$^+$. $\log \beta_{110} = 0$; Sr(II).asn$^+$, $\log \beta_{110} = -0.43$, and Schubert observed that in general amino-acids do not bind appreciable fractions of the alkaline earths in the physiological pH region.

In the late 1950's conflicting results for the same system, namely copper(II)-asparaginate, were obtained by two American research groups, Li et al[138] and Bennett[169]. Li et al found that the copper-asparaginate complexes had the same formation constants regardless of whether the ligand was in the D-, L- or racemic form. On the other hand, Bennett found the formation constant for the complex Cu(II). L-asn_2 to be about four times as large as the formation constant for the complex Cu(II).DL-asn_2.

Ritsma et al undertook a re-investigation of this phenomenon, and their results agreed with the findings of Li et al, showing that there was absence of stereospecificity in the formation of metal asparaginate complexes, attributed to the small influence (steric or otherwise) the ligands exert on each other. Stereospecificity has however been shown to exist in other amino-acid systems, for example Co(II).D-his.L-his. is more stable than either Co(II).D-his_2 or Co(II).L-his_2[170].

At about the same time as Li et al and Bennett published their differing results on copper-asparaginate complexation Perrin made a re-investigation of the complexation of amino-acids with Fe(III), work which had previously been carried out by Albert[70], and found that, far from not forming any complexes with amino-acids as Albert had reported, Fe(III) bound amino-acids even more strongly than Cu(II)[152]. Results for the Fe(III) phenylalaninate system published by Williams confirm this observation[28].

In the 1960's there was a series of studies of amino-acid complexation with a wide variety of cations. Chromium(III) forms a large number of octahedral complexes, which have attracted the attention of many kineticists : indeed Cr(III) complexes are second only to Co(III) complexes in importance as substrates for exploring aspects of substitution reactions in solution, e.g. acid and base hydrolysis[171]. However the literature records relatively few stability constant measurements, particularly for organic ligand complexes of Cr(III). This situation may now be altered by the fact that chromium has been shown to be beneficial to health (see

chapter 1, also reference 7). Khan and Malik[172] have made measurements of stability constants for complexes of selected amino-acids, including asparaginate, with Cr(III). Their results for Cr(III)-asparaginate were : $\log \beta_{110} = 7.7$, $\log \beta_{210} = 13.6$, $\log \beta_{310} = 18.5$ (at $25^{o}C$, I = 0.5M), i.e. these Cr(III) complexes are nearly as stable as the corresponding Cu(II) complexes.

Rao and Subramanya[161] measured stability constants for complexes between asparaginate and Cd(II) and Pb(II), by polarography and obtained, for the first time, constants for hydroxy species $Cd.asn_3.OH^{2-}$ and $Pb.asn._2.OH^{-}$. Subsequent work on the same metal ions with the same amino-acid by Williams et al[173-174] recorded only the simple BA_n species where n could be 1, 2 or 3. This was the first occasion that tris complexes of amino-acids with lead had been detected in solution. Williams and Graham[121] also discovered a species $Pd(II).asn.H^{2+}$ ($\log \beta = 12.11$) while investigating ternary complex formation between palladium(II), asparaginate and chloride ions : the first occasion that a protonated metal asparaginate complex had been reported.

Another ion whose complexes with amino-acids have been studied in recent years is silver(I), by Azizov et al[160]. For asparaginate $\log \beta_{110} = 3.30$ and $\log \beta_{210} = 6.45$, and the amino-acid co-ordinates only through the amine nitrogen atom, in accordance with the preference for silver(I) for a co-ordination number of two, in a linear configuration. This applies to all amino-acids except methioninate, which is large enough to form a

bidentate complex, through N and S, with a stability constant three log units higher than any other Ag(I)-amino-acid complex.

After the present work was begun, Gergely et al[137] published some work on copper(II)– asparaginate in which they found that at pH 11 a proton comes off the amide group in the AB and A_2B complexes. This only occurs in the presence of the metal ion, and it allows the amide group to co-ordinate axially, thus creating a tridentate ligand.

This completes the review of all previous measurements of stability constants for metal-asparaginate complexes. Measurements of heats of formation for these same complexes, and for metal-amino-acid complexes in general, are far less numerous. Glycinate, being the simplest amino-acid, has more ΔH^o measurements for its metal complexes than any other amino acid. A number of values of ΔH^o for copper(II)-amino-acid complexes exist, measured by Izatt et al[90,135] using calorimetry, and there are also some ΔH^o values of Co(II), Ni(II), Cu(II) and Zn(II) complexes with amino-acids obtained by Stack and Skinner[175], using microcalorimetry. The two literature values for formation of Cu(II)-asparaginate complexes are by Barnes and Pettit[143], who used the stability constants of Ritsma et al[31] in their calculations, and more recently by Gergely et al[137]. These literature values have already been quoted in Chapter 6. ΔH^o values for formation of complexes of other metal ions with amino-acids, excluding asparaginate, have also been obtained by the less reliable van't Hoff isochore method[142], where log β values are measured

at three or more different temperatures and ΔH^{o} is then obtained from plots of $\log\beta$ against $^{1}/T$. In this approach ΔH^{o} is assumed not to vary with temperature, an unsound assumption.

In addition to this thermodynamic data, some structural and spectral data for metal asparaginate complexes exists in the literature. This will be discussed in a later section.

Summary of results.

Table 33 lists the ΔG^{o}, ΔH^{o} and ΔS^{o} values for metal-asparaginate complexes obtained in the present work. The $\Delta_{0,x}$ terms are overall constants and $\Delta_{1,2}$ and $\Delta_{2,3}$ terms are stepwise constants. Figures in parentheses are three times the standard deviations in the computed constants, and n is the number of calorimetric measurements.

A few general points will be mentioned before an analysis of the results is undertaken.

The increasing dominance of the (II) oxidation state as one crosses the first transition series is an important aspect of transition metal chemistry. This increased stability to the eventual exclusion of all other oxidation states at zinc arises because the 3d orbitals change from being diffuse excited orbitals into tightly bound core orbitals. The (II) oxidation state becomes most important from manganese onwards and except for Cu(I) and Fe(III) the aqueous chemistry of these transition metals is entirely that of the (II) state. However Fe(III) merits special attention, not only for its aqueous chemistry but also for its biological importance. This (III) state for iron occurs against a trend of decreasing occurrence of oxidation states

Table 33. Thermodynamic parameters for the formation of complexes between asparaginate and metal ions at 25° in 3.00M(Na)ClO_4

ΔG° and ΔH° are expressed in kJ mol^{-1} and ΔS° in J mol^{-1} K^{-1}.

	H^+	Mn(II)	Fe(II)	Co(II)	Ni(II)	Cu(II)	Zn(II)
$-\Delta G^\circ_{0,1}$	53.10 [0.10]	17.70 [0.23]	24.93 [0.19]	28.00 [0.05]	35.12 [0.04]	49.54 [0.13]	28.93 [0.02]
$-\Delta G^\circ_{1,2}$	14.76 [0.13]	12.10 [0.32]	18.28 [0.19]	23.56 [0.05]	28.66 [0.04]	41.10 [0.13]	24.87 [0.02]
$-\Delta G^\circ_{0,2}$	67.86 [0.13]	29.80 [0.55]	43.21 [0.21]	51.56 [0.06]	63.78 [0.06]	90.64 [0.14]	53.82 [0.02]
$-\Delta G^\circ_{2,3}$			15.37 [0.21]	18.12 [0.25]	19.26 [0.25]		16.26 [0.13]
$-\Delta G^\circ_{0,3}$			58.58 [0.31]	67.68 [0.31]	83.04 [0.31]		70.18 [0.15]
$-\Delta H^\circ_{0,1}$	50.50 [0.40]	7.26 [0.75]	Study prevented because of oxidation of Fe(II) overnight in calorimeter	11.95 [0.50]	17.11 [0.40]	27.5 [1.0]	10.44 [0.40]
$-\Delta H^\circ_{1,2}$	5.10 [0.05]	6.98 [0.75]		14.76 [0.50]	26.34 [0.40]	34.0 [1.0]	12.73 [0.40]
$-\Delta H^\circ_{0,2}$	55.60 [0.40]	14.23 [1.50]		26.71 [1.00]	43.45 [0.80]	61.5 [2.0]	23.17 [0.80]
$-\Delta H^\circ_{2,3}$				9.69 [1.00]	20.05 [0.80]		4.38 [0.80]
$-\Delta H^\circ_{0,3}$				36.40 [1.50]	63.50 [1.20]		27.55 [1.20]

Table 33 continued overleaf.

	H^+	Mn(II)	Fe(II)	Co(II)	Ni(II)	Cu(II)	Zn(II)
$\Delta S^\circ_{0,1}$	8.9 [1.0]	35.0 [1.90]	Study prevented because of oxidation of Fe(II) overnight in calorimeter	53.8 [1.8]	60.4 [1.2]	73.9 [2.9]	62.1 [1.3]
$\Delta S^\circ_{1,2}$	32.4 [0.6]	17.2 [1.90]		29.5 [1.8]	7.5 [1.8]	23.8 [3.3]	40.6 [1.3]
$\Delta S^\circ_{0,2}$	41.3 [1.0]	52.2 [3.50]		83.3 [3.2]	67.9 [2.5]	97.7 [6.2]	102.7 [2.6]
$\Delta S^\circ_{2,3}$				21.6 [3.2]	-2.4 [2.5]		40.3 [2.6]
$\Delta S^\circ_{0,3}$				104.8 [4.0]	65.5 [2.3]		143.0 [4.5]
number of observations. n	42	16		29	28	29	19

Table 34 shows the results obtained for glutaminate complexing.

Table 34. Thermodynamic parameters for the formation of complexes between glutaminate and metal ions at 25° in 3.00M(Na)ClO_4

	H^+	Ni(II)	Cu(II)
$-\Delta G^\circ_{0,1}$	55.03 [0.02]	31.75 [0.05]	51.68 [0.01]
$-\Delta G^\circ_{1,2}$	15.55 [0.02]	26.96 [0.06]	42.76 [0.34]
$-\Delta G^\circ_{0,2}$	70.58 [0.02]	58.71 [0.11]	94.46 [0.44]
$-\Delta G^\circ_{2,3}$		20.17 [0.19]	
$-\Delta G^\circ_{0,3}$		78.88 [0.30]	
$-\Delta H^\circ_{0,1}$	50.86 [0.50]	13.28 [0.60]	16.5 [1.0]
$-\Delta H^\circ_{1,2}$	4.42 [0.50]	22.81 [0.60]	26.0 [1.0]
$-\Delta H^\circ_{0,2}$	55.28 [1.00]	36.09 [1.20]	42.5 [2.0]
$-\Delta H^\circ_{2,3}$		18.66 [1.20]	
$-\Delta H^\circ_{0,3}$		54.75 [1.80]	
$\Delta S^\circ_{0,1}$	14.0 [1.6]	61.9 [1.80]	118.0 [3.0]
$\Delta S^\circ_{1,2}$	37.4 [1.7]	18.0 [1.90]	56.0 [3.0]
$\Delta S^\circ_{0,2}$	51.4 [3.3]	79.9 [3.70]	174.3 [5.2]
$\Delta S^\circ_{2,3}$		5.0 [3.70]	
$\Delta S^\circ_{0,3}$		84.9 [5.00]	
number of observations. n	35	24	30

greater than (II) because of the comparatively low third ionization potential for iron, which in turn arises from the metal's electronic configuration, and the stability of Cu(I) can be explained in similar terms.

The (II) oxidation state occurs for the metals manganese through to zinc by ionization of the two 4s electrons to produce an outer electronic structure of $3d^n$ where n runs from 5(manganese) up to 10(zinc). All the ions form complexes and are hydrated in aqueous solution. Small ligands usually produce octahedral complexes and so the aquated ions are written $B(H_2O)_6^{2+}$. Two exceptions are copper, which forms John-Teller distorted octahedral complexes, becoming square planar in extreme cases[176], and zinc, which forms a large number of tetrahedral complexes in addition to octahedral complexes.

The present work has reported complexes with three amino-acids per central metal ion, this occurring for all metal ions except manganese(II) and copper(II). Precipitation occurred in the manganese system around $\bar{Z} = 2$, possibly due to formation of an insoluble complex, $A_2B.2H_2O$. Complexes of this stoichiometry between amino-acids and cobalt or nickel have been reported previously by other workers[119,177]. For ions in the series Mn(II) through to Zn(II) the general order of stability constants, and hence ΔG^o values, for the replacement of water by more polarisable ligands to form octahedral complexes is given by the Irving-Williams series[178].

$$Mn < Fe < Co < Ni < Cu > Zn.$$

Other series (e.g. Maley and Mellor[179]) which include other M(II) ions have been proposed, but have only limited validity, for they do not remain the same upon changing the ligand. The order Mn < Fe < Cd < Co < Zn < Ni < Cu is valid for almost all amino-acids, but fails for 8-hydroxy-quinolinate, where iron and cadmium must be interchanged. With nitroacetate, cadmium and cobalt are out of place, and with salicylate and histidinate, cobalt and zinc change places. The Irving-Williams order seems to hold without exception, and its theoretical justification is based on consideration of the reciprocals of the ionic radii and the second ionisation potentials of the metals concerned[178].

As can be seen from Table 33, the results obtained in this study obey the Irving-Williams order. It is also apparent that, having accepted that concentration constants in 3.00M(Na)ClO_4 are larger than those at lower ionic backgrounds, the results are in agreement with those published by other workers. This present work is the first to list thermodynamic parameters for tris complexes between metal ions and asparaginate ; in a number of previous studies there are no constants given for the simple AB species (see Table 10 in Chapter 5). As asparaginate is a biologically important ligand (see Chapter 1) it is remarkable that so few constants have been published for its complexes with metal ions.

For all the metal ions studied $-\Delta H^{o}_{0,1}$ is less than $-\Delta H^{o}_{1,2}$, an effect which has previously been reported by Izatt et al[90] and ascribed to the

large difference in hydration energies between $B^{2+}_{(aq)}$ and $AB^{+}_{(aq)}$. On the other hand $\Delta S^{o}_{0,1}$ is consistently larger than $\Delta S^{o}_{1,2}$, which is mainly due to statistical factors. The overall effect is that $-\Delta G^{o}_{0,1}$ is greater than $-\Delta G^{o}_{1,2}$, a fact uniformly true for all literature values of metal-amino-acid stability constants, with the solitary exception of Sychev and Migal's phenylalaninate work[180], where the experimental approach has been queried by Gergely et al[177].

The zinc system is marginally more stable than the cobalt, a trend noted for all amino-acid systems except histidinate[26]. The formation curves for zinc and cobalt (figure 5) cross at $\bar{Z} = 2.2$ because of a low $\Delta H^{o}_{2,3}$ for $Zn(II).asn_3^{-}$, which could be the result of zinc's preference for tetrahedral co-ordination. The same reasons could be put forward for the large entropies for the zinc complexes.

$\Delta S^{o}_{2,3}$ values for the nickel systems are surprisingly low, a pattern not found in previous amino-acid studies in this laboratory. This conceivably arises because the amide groupings occupy, albeit without formal bonding, co-ordination positions in bis complexes, so that when the third ligand is added very little water of solvation needs to be removed from these positions. In this connection ΔS^{o} values for the bis complexes are higher than expected for comparable systems, i.e. three metal-bond positions are already desolvated. Further work is necessary to establish why this trait is so particular to nickel complexes.

An interesting point arises from this and other work on the copper(II)

systems, whether the ligands are bidentate or tridentate to copper(II). Table 35 lists $\Delta H^{o}_{0,1}$ and $\Delta S^{o}_{0,1}$ for the copper(II) complexes of the ligands considered in this work, and related reference ligands.

Table 35. Values of $\Delta H^{o}_{0,1}$ (kJ mol^{-1}) and $\Delta S^{o}_{0,1}$ (JK^{-1} mol^{-1}) for forming 1:1 complexes with Cu(II), and suggested number of bonds from the ligand to the metal ion.

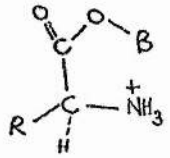
Complex	$-\Delta H^{o}_{0,1}$	$\Delta S^{o}_{0,1}$	Bonding	Reference
Cu(II).his^{+}	43.9	45.9	Tridentate	27
Cu(II).asn^{+}	27.5	73.9	Bi-or tri-dentate	This work
Cu(II).phe^{+}	19.2	93.7	Bidentate	28
Cu(II).thr^{+}	18.0	104.2	Bidentate	This work
Cu(II).gln^{+}	16.5	118.0	Bidentate	This work

Phenylalaninate(phe) has only two donor groups (NH_2 and CO_2^-) and so can only be bidentate. Its $\Delta H^{o}_{0,1}$ and $\Delta S^{o}_{0,1}$ for complex formation are similar to those for threoninate and glutaminate, which are therefore assumed to be bidentate also. Histidinate, on the other hand, is customarily tridentate to octahedral metal ions[26] and its somewhat strained tridentate nature in Cu(II) complexes has already been the topic of intense investigation[27]. The increased ligand-metal bond strengths arising from two chelate rings are shown by the heat of formation for the 1:1 histidinate-copper(II) complex being double that for any of the bidentate examples. Further, the reduced degrees of freedom when the two chelate rings are formed

correspondingly reduces the entropy of formation by half that of the bidentates. Copper(II)-asparaginate has $\Delta H^{o}_{0,1}$ and $\Delta S^{o}_{0,1}$ values that are intermediate between bi- and tri-dentate complexing. This suggests that the amide group of the ligand is either weakly localised in the vicinity of one of the long axial bonds of Cu(II) or that there is a mixture of bi- and tri-dentate asparaginate complexes present, the calorimetric results being the average of the two.

The most recent metal-amino-acid publications have included various protonated and hydrolysed species. While the major complexes formed are the A_nB species, protonated, hydrolysed and polynuclear species have been found to exist for ligands related to amino-acids and a few amino-acids themselves[27,181]. Perrin[135] has obtained constants for protonated species ABH and A_2BH involving a series of divalent metal ions and amino-acids. These complexes were said to be analogous to acetate complexes and hence the amine group was protonated and the metal-ligand bond utilised the carboxyl group. Jones and Williams[182-183] have reported the formation of protonated species with the lanthanide(III)ions and histidinate, where the protonation is on the imidazole nitrogen[163].

In the present study no protonated species were detected. The formation of such complexes as



is dependent on the type of R group and so for aliphatic groups which are electron repelling e.g. $-CH_3$, the positive charge on the nitrogen will be stabilised, whereas for electron withdrawing groups such as aromatic rings the charged nitrogen will be destabilised, and hence protonation will be inhibited and complex formation will be encouraged.

The maximum $\bar{Z}$ measurable for all systems was determined by the solubility of the hydrolysis products of the complexes formed. However, in general, solubility was not a major problem in this study, asparagine being soluble up to 160mM and its complexes not being markedly less soluble. Hydrolysis of these complexes resulted in an immediate visible precipitation, but these hydrolysed species could not be detected potentiometrically as β values for the equilibrated solutions, and the precipitates could not be isolated and identified because of their gelatinous nature.

(c) The homologue "paradox".

In Chapter 6 the so-called homologue "paradox" was examined and conclusions may be drawn from the associated experimental data.

The effect occurs in glutaminate-asparaginate (i) for ΔG^o protonation values at all ionic strengths, (ii) for ΔG^o values for Mn(II), Co(II), Ni(II) and Zn(II) (but not for Cu(II)) complexes with the ligands (see tables 33, 34, also references 29 and 136), (iii) for ΔH^o values for Ni(II) and Cu(II) complexes but it is not noticeable among ΔS^o values.

The amino-acid protonation versus complexation homologue paradox

appears to arise from two factors (i) aquation of ligand and cation and (ii) chelation occurring during metal complexing. The present postulates are that metal complexation liberates more water of solvation than does protonation and that chelation brings the non-bonding chain and amide group on the ligand into the hydration sphere of the metal ion, i.e. the number of $-CH_2$ groups in this side chain influences the extent of metal ion desolvation. The following evidence exists for this :- For nickel(II) and copper(II) ΔH^o values, asparaginate-B(II) either has stronger bonding than glutaminate -B(II) or less solvation water -B(II) bonds need to be broken for complexation to occur. Comparing the ΔS^o values, the asparaginate systems either have more constrictions involved in their complexes, or less water molecules are lost. For asparaginate and glutaminate protonation ΔH^o values, bonding is essentially equal, i.e. essentially the same number of water-ligand bonds are broken per carboxylate site, regardless of whether it is on asparaginate or glutaminate. ΔS^o values indicate that the number of water molecules librated per protonation to be marginally more for glutaminate.

(d) The complexing reactions of individual metal ions.

(i) Manganese(II)

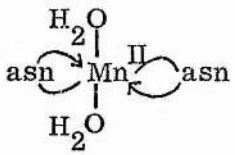
For manganese the (II) state is the most important and generally the most stable oxidation state. In neutral or acid aqueous solution it exists as the very pale pink hexaquo ion $Mn(H_2O)_6^{2+}$ which is quite resistant to oxidation. In basic media, however, the white hydroxide $Mn(OH)_2$ is formed and rapidly goes brown in air because of oxidation to Mn(III).

$$Mn(OH)_2 \xrightarrow[\text{oxidation}]{\text{air}} Mn_2O_3\,xH_2O$$

The (III) state can also be produced by electrolytic or persulphate oxidation of Mn(II) solution. It cannot be obtained in high concentrations because it is reduced by water, even in the presence of complexing ligands such as EDTA[184].

Manganese(II) forms many complexes, but the formation constants for these in aqueous solution are lower than those for the divalent cations of succeeding elements, because Mn(II) is larger than any of the other cations in the Irving-Williams series, and neither the hexaquo ion nor most of the Mn(II) complexes, which are high spin, have any ligand field stablisation energy. Chelating ligands such as ethylenediamine, EDTA, oxalate ions, etc, form complexes isolable from aqueous solution. For a ligand such as asparaginate one might expect to obtain simple complexes up to BA_3^-, but precipitation occurred below $\bar{Z} = 2$ so that only BA^+ and BA_2 were detected. The insolubility of manganese systems has been noted by other workers[29].

The structure of the complex Mn(II).asn_2 is thought to be



The u.v./visible spectra of Mn(II) octahedral complexes in aqueous solution are characteristically weak. The reason for this is that the ground state of the d^5 system in a weak octahedral field has one electron in each d

orbital and their spins are parallel, making a spin sextuplet, corresponding to the ^6S ground state of the free ion, which is not split by the ligand field. However this is the only sextuplet state possible, for every conceivable alteration of the electron distribution $t_{2g}^3 e_g^2$ results in the pairing of two or four spins, thus making quartet or doublet states. Hence all excited states of the d^5 system have different spin multiplicities from the ground state, and transitions to them are spin-forbidden. Because of weak spin-orbit interactions such transitions are not totally absent, but the resultant absorption bands are roughly 100 times weaker than those for similar but spin-allowed transitions.

Mn(II) also forms a number of tetrahedral complexes, such as $MnBr_4^{2-}$. They are yellow-green in colour, the colour being more intense than that of the octahedral complexes.

(ii) Iron(II).

In the absence of other complexing agents, aqueous solutions of Fe(II) contain the pale blue-green hexaquo ion $Fe(H_2O)_6^{2+}$. The potential of the important Fe(III)-Fe(II) couple, 0.771V, is such that molecular oxygen can convert ferrous into ferric ions in acid solution.

$$2Fe(II) + \tfrac{1}{2}O_2 + 2H^+ \longrightarrow 2Fe(III) + H_2O$$

In basic media the oxidation process is still more favourable. Ferrous hydroxide, which is white, goes brown in air almost immediately after being formed. The brown material is a hydrated ferric oxide. It is

therefore important to rigorously exclude air from experiments involving Fe(II). Neutral and acid solutions of Fe(II) oxidise less rapidly with increasing acidity (despite the fact that the potential of the oxidation reaction becomes more positive), because Fe(III) is actually present in the form of hydroxy complexes, except in extremely acid media, and there may also be kinetic reasons.

The oxidation of Fe(II) to Fe(III) in neutral solution has been the subject of much speculation and may involve a reaction between $FeOH^+$ and HO_3^- [185]. The related problem of oxidation of Fe(II) with H_2O_2 is complicated and involves radicals generated by the reaction[186].

$$Fe(II) + H_2O_2 \longrightarrow Fe(III)OH\cdot + OH\cdot$$

Iron(II) forms a number of complexes, mostly octahedral. Ferrous complexes can normally be oxidised to ferric complexes and the Fe(II)-Fe(III) aqueous system provides a good example of the effect of complexing ligands on the relative stabilities of oxidation states.

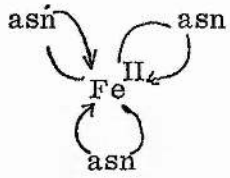
e.g. $Fe(CN)_6^{3-} + e \longrightarrow Fe(CN)_6^{4-}$ $E^o = 0.36V$

$Fe(H_2O)_6^{3+} + e \longrightarrow Fe(H_2O)_6^{2+}$ $E^o = 0.77V$

$Fe(phen)_3^{3+} + e \longrightarrow Fe(phen)_3^{2+}$ $E^o = 1.12V$

With chelating amine ligands, many complexes stable in aqueous solution are known. Ethylenediamine forms $Fe(en)^{2+}$, $Fe(en)_2^{2+}$ and $Fe(en)_3^{2+}$ [187], and in the present work it was discovered that three

asparaginate ligands could bind to one Fe(II) ion :



Although the results obtained from the Fe(II)-asparaginate system are limited they are more comprehensive than any comparable study published by other schools for the same amino-acid system. Logical extensions of this work are (i) to study the Fe(III)-asparaginate system, where hydrolysed species are a major feature and have been reported for other amino-acids[28], (ii) to measure redox potentials for a range of Fe(II)/Fe(III) complexes in 3.00M(Na)ClO_4 and thus describe the Fe(II)/Fe(III)-asparaginate system and (iii) design a system which would enable the calorimetric investigation of Fe(II)-amino-acid complexation to be carried out.

The Fe(II)-Fe(III) couple has been extensively studied in the presence of a wide variety of ligands, most recently by Irving and Sharp[188]. The multitude of complexes which occur in these systems demand a computer and a specially designed program to calculate the results.

(iii) Cobalt(II).

The trend towards decreased stability of high oxidation states, and the increased stability of the (II) state relative to the (III) state persists with cobalt, where there is complete absence of oxidation states higher than (IV) under chemically significant conditions, and even (IV) is highly uncertain, being represented by only a few incompletely characterised compounds such

as Cs_2CoF_6 and CoO_2.

In aqueous solutions containing no complexing agents, the oxidation of Co(II) to Co(III) is very unfavourable.

$$Co(H_2O)_6^{2+} \longrightarrow Co(H_2O)_6^{3+} + e \quad E^o = -1.84V$$

However electrolytic or ozone oxidation of cold perchloric acid solutions of Co(II) gives dark blue-green solutions of $Co(H_2O)_6^{3+}$ which is in equilibrium with $Co(OH)(H_2O)_5^{2+}$. At 0^oC the half life of these diamagnetic aquo ions is about a month[189]. In the presence of complexing agents, such as ammonia, which forms stable complexes with Co(III) the stability of trivalent cobalt is greatly improved.

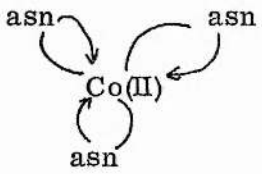
$$Co(NH_3)_6^{3+} + e \longrightarrow Co(NH_3)_6^{2+} \qquad E^o = 0.10V$$

Co(II) forms numerous complexes of various stereochemical types. Octahedral and tetrahedral ones are the most common, but there are a number of square ones as well as some which are five-co-ordinate[190]. Co(II) forms tetrahedral complexes more readily than any other metal ion. This is in accord with the fact that for a d^7 ion, ligand-field stabilisation energies disfavour the tetrahedral configuration relative to the octahedral one to a smaller extent for a d^7 ion than for any other d^n configuation. It may be noted that cobalt(II) is the only d^7 ion of common occurance.

Because of the small difference in stability between octahedral and tetrahedral Co(II) complexes there are several instances in which the two types are both known and may be in equilibrium. The existence of some

$Co(H_2O)_4^{2+}$ in equilibrium with $Co(H_2O)_6^{2+}$ has been proved[191].

In the present study Co(II) was found to bind a maximum of three asparaginate ligands.



The only previous reference in the literature to the complex $Co(II).asn_3^-$ is by Tanford and Shore, who noted that the structure of the chelating group was "abnormal"[30].

Complexes of Co(II) with amino-acids, including asparaginate, react with molecular oxygen to form Co(III) species, reactions which are not entirely reversible[192].

The biological importance of cobalt has not been fully characterised, and much more work, both in vivo and in vitro, is necessary before the full implications of the Co(II)-Co(III) couple and the unusual stereochemistries of the complexes are determined and understood.

(iv) Nickel(II).

The trend towards decreased stability of higher oxidation states continues with nickel, so that only Ni(II) occurs in the ordinary chemistry of the element. Even in the few compounds formally containing Ni(III) and Ni(IV) there is doubt about the physical significance of these oxidation numbers. Lower-valent nickel is also uncommon, except in compounds containing strongly π -bonding ligands. However, the relative simplicity

of nickel chemistry in terms of oxidation number is balanced by considerable complexity in co-ordination numbers and geometries.

Nickel(II) forms a large number of complexes encompassing co-ordination numbers 4, 5 and 6, and all the main structural types, square, tetrahedral, square-pyramidal, trigonal-bipyramidal and octahedral, are represented. It is characteristic of Ni(II) complexes that complicated equilibria, which are generally temperature-dependent, and sometimes also concentration-dependent, often exist between these structural types.

The maximum co-ordination number for Ni(II) is six. A large number of neutral ligands, especially amines, displace some or all of the water molecules in the octahedral $Ni(H_2O)_6^{2+}$ ion to give characteristically blue or purple colours in sharp contrast to the bright green of the hexaquo ion. This occurs because of shifts in the absorption bands when water ligands are replaced by others lying towards the stronger end of the spectrochemical series.

A considerable number of both trigonal-bipyramidal and square pyramidal complexes of Ni(II) are known[193]. Many of those contain tetradentate "tripod" ligands such as $N[CH_2CH_2N(CH_3)_2]_3$, and the fifth co-ordination position is occupied by ligands such as Cl^-, Br^- or I^-, so these complexes are cationic. Other 5-co-ordinate complexes include $Ni(CN)_5^{3-}$.

The tetrahedral Ni(II) complexes are of types such as NiX_4^{2-} and NiX_3L^-, where X represents a halogen and L is a neutral ligand such as a

phosphine or arsine. Planer complexes include $Ni(CN)_4^{2-}$, and the red dimethylglyoxime complex.

The structures of the Ni(II)-asparaginate complexes, which were deep blue in alkaline solution, are likely to be similar to those of the corresponding cobalt complexes. This work is the first to report the formation of the complex $Ni(II).asn_3^-$.

The complex $Ni(II).asn_2$ has been isolated as blue crystals from a mixture of nickel carbonate and the amino acid[194]. In aqueous solution three absorption bands were observed in the visible /u.v. spectrum, at $9810cm^{-1}$, $16340cm^{-1}$ and $27930cm^{-1}$, with extinction coefficients 6.92, 4.60 and 12.8. In addition a weak band ($\epsilon = 2.0$) was seen at $13000cm^{-1}$. These findings are in accordance with octahedral geometry.

Cassatt and Wilkins[195] have measured the rate constants for the formation of the mono complexes of nickel(II) with various ligands, including asparaginate, by the stopped-flow method. They observed that the zwitterionic form of the ligand was unreactive, and postulated the reason as being that in this case the first step of co-ordination occurs through the $-CO_2^-$ group. This is a labile arrangement and the breaking rate constant would be larger than the combined process of H^+ ionisation from $-NH_3^+$ and ring closure. This unfavourable situation does not occur with the species $NH_2CH_2CO_2^-$ which exists at higher pH. The observed rate constant (k_{obs}) for formation of $Ni(II).asn^+$ was $110M^{-1}s^{-1}$ at pH 7.0, but only $14M^{-1}s^{-1}$ at pH 6.0 (I = 0.3M). The calculated rate constant k_1 was $8.7 \times 10^3 M^{-1}s^{-1}$.

(v) Copper(II).

The (II) state is dominant in the aqueous solution chemistry of copper because of the element's low second ionisation potential, and the high energy of hydration for the cupric ion. A large number of salts of various anions many of them water-soluble, exist in addition to a wealth of complexes.

The d^9 configuration makes Cu(II) subject to Jahn-Teller distortion if placed in a regular octahedral or tetrahedral environment, and this has a profound effect on its stereochemistry. When six-co-ordinate the octahedron is severely distorted along one four-fold axis so that there is a planar array of four short B--A bonds and two trans long ones, e.g. in crystalline $CuCl_2$, four Cu-Cl bonds are 2.30Å long and the other two are 2.95Å. In the limit, the elongation leads to a situation indistinguishable from square co-ordination as found in CuO and many discrete complexes of Cu(II). Thus the cases of tetragonally distorted "octahedral" co-ordination and square co-ordination cannot be sharply differentiated. There is just one complex, $K_2Pb[Cu(NO_2)_6]$, where the octahedron of nitrogen atoms surrounding the Cu(II) ion is regular[196]. It is not known why the Jahn-Teller distortion is too small for detection in this case.

In addition to the normal square merging into tetragonally distorted octahedral complexes, there are other stereochemistries of which the most important is distorted tetrahedral. Cs_2CuCl_4 is an example of a distorted tetrahedral complex.

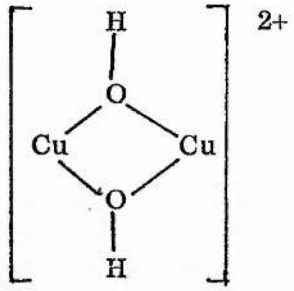
Most cupric salts dissolve in water to give the aquo ion, which may

be written $Cu(H_2O)_6^{2+}$. This reacts with various ligands to form complexes. With ammonia the species $Cu(NH_3)(H_2O)_5^{2+}$ up to $Cu(NH_3)_4(H_2O)_2^{2+}$ are formed in the usual way, but addition of the fifth and sixth molecule of ammonia is very difficult[197]. The reason for this unusual behaviour is connected with the Jahn-Teller effect. Because of it, the Cu(II) ion does not bind the fifth and sixth ligands strongly (even the H_2O). Similarly it is found with ethylenediamine that $Cu\ en(H_2O)_4^{2+}$ and $Cu\ en_2(H_2O)_2^{2+}$ form readily, but $Cu\ en_3^{2+}$ is formed only at extremely high concentrations of en.

Many other amine complexes of Cu(II) are known and all are much more intensely blue than the aquo ion, because the amines produce a stronger ligand field, which causes the absorption band to move from the far red to the middle of the red region of the spectrum. For example, the aquo ion has an absorption maximum at $12500cm^{-1}$, whereas in $Cu(NH_3)_4(H_2O)_2^{2+}$ the maximum is around $16500cm^{-1}$.

The formation of $Cu(asn)^+$ and $Cu(asn)_2$ only is to be expected under the present conditions. Like the ammonia complexes, they are deep blue in solution. As already mentioned, $\triangle H^o$ values for the asparaginate complexes indicate that the amide grouping may occupy an axial co-ordination position on the metal ion, so giving rise to a tridentate ligand.

In the presence of oxygen donors, Cu(II) forms a series of poly-nuclear species, the simplest of which is the hydroxy complex reported by Biedermann[89].



Various workers have reported complexes of formula $A_2B_2(OH)_2$ where the range of ligands A includes phenylalaninate[28], histidinate[154] and acetate[176]. The present work failed to find any evidence of such complexes for asparaginate. However interpretation of the biological role of copper and its compounds ought to take into account the presence of hydrolysed species, if we are to learn from the importance of hydrolysed iron(III) species in liver storage.

A certain amount of other experimental work on Cu(II)-asparaginate complexes is described in the literature. Wilson et al[55] examined the circular dichroism and absorption spectra of Cu.asn$_2$. They discovered that Cu.asn$_2$, exhibited CD and absorption spectra similar to Cu(II)-alaninate complexes at pH7 (ν max = 15800cm^{-1}, ϵ = 45), but on addition of excess base an amide nitrogen was found to ionise, and some precipitation took place. The circular dichroism changed sign in the same manner as Cu(II) histidinate, and it was suggested that the amide nitrogen bound to copper leaving the carboxylate oxygen occupying an apical co-ordination position (c.f. Cu(II).his$^+$). O.R.D. work by Wellman et al[198] and by Jursik and Hajik[199] supports this idea.

Fujimoto[200] has studied the crystallisation of asparagine monohydrate crystals from very dilute $CuCl_2$ solutions and has obtained a trans bis-asparaginate Cu(II) dihydrate, in which the copper ion is co-ordinated by two $-NH_2$ groups, two CO_2^- groups and two axial water molecules. The complex structure characterised by the ligand nitrogen hyperfine structure in EPR spectra has been found to depend on the pH of the solution from which the crystals are grown.

(vi) Zinc(II).

Zinc follows copper in the periodic table and has two s electrons outside the filled d shell, but whereas in copper the d shell may lose an electron to give ions or complexes, this is not possible for zinc and there is no evidence for oxidation states higher than (II).

Since zinc forms no compound in which the d shell is missing an electron it is regarded as a non-transition element, whereas copper does and is therefore a transition element. Zinc is a more ductile metal and has a lower melting point than copper (Zn 419°C; Cu, 1083°C)and is more electropositive. However there is some resemblence to the d-block elements in the ability to form complexes, especially with ammonia, amines, halide ions and cyanide ions.

Zinc salts of oxo acids such as the nitrate, sulphate and perchlorate dissolve in water to give the aquo ion which is quite a strong acid, and aqueous solutions of these salts are hydrolysed[201]. In perchlorate solution the only hydroxy species for zinc below 0.1M is of the formula $Zn(OH)^+$.

Addition of alkali precipitates the hydroxide $Zn(OH)_2$, which redissolves in excess alkali to form the zincate ion $Zn(OH)_4^{2-}$ [202]. Salts such as $Na_2Zn(OH)_4$ can be crystallised from concentrated solutions.

In its complexes zinc commonly has co-ordination number of 4, 5 and 6. Tetrahedral examples are the halide complexes ZnX_4^-, which are of rather low stability. A number of five-co-ordinate complexes have recently been found, including the hydrazinecarboxylate complex $Zn(NH_2NHCOO)_2$ [203]. Since there is no ligand-field stablisation energy for zinc, the stereochemistry is determined by size, electrostatic effects and covalent bonding forces.

In the present study complexes up to $Zn(asn)_3^-$ were detected in solution, but $\triangle H^o$ values suggest that Zn(II) would prefer to be four-co-ordinate rather than six-co-ordinate.

Simple model of asparaginate in blood plasma.

The version of COMICS loaded in the computer at St. Andrews was used to compute the concentrations of metal asparaginate complexes in blood plasma for a range of pH values. The total metal ion concentrations used were blood plasma exchangeable values, e.g. the copper concentration was albumin and amino-acid bound, not the non-exchangeable ceruloplasmin copper concentration, and the formation constants were as in Chapter 5. The results shown in figure 20 suggest that the complexes $Zn.asn.^+$, $Fe.asn.^+$ and $Co.asn.^+$ may be present in concentrations comparable to $Cu.asn.^+$ and $Cu.asn._2$. This situation occurs in spite of the copper complexes having the highest formation constants because exchangeable copper is found

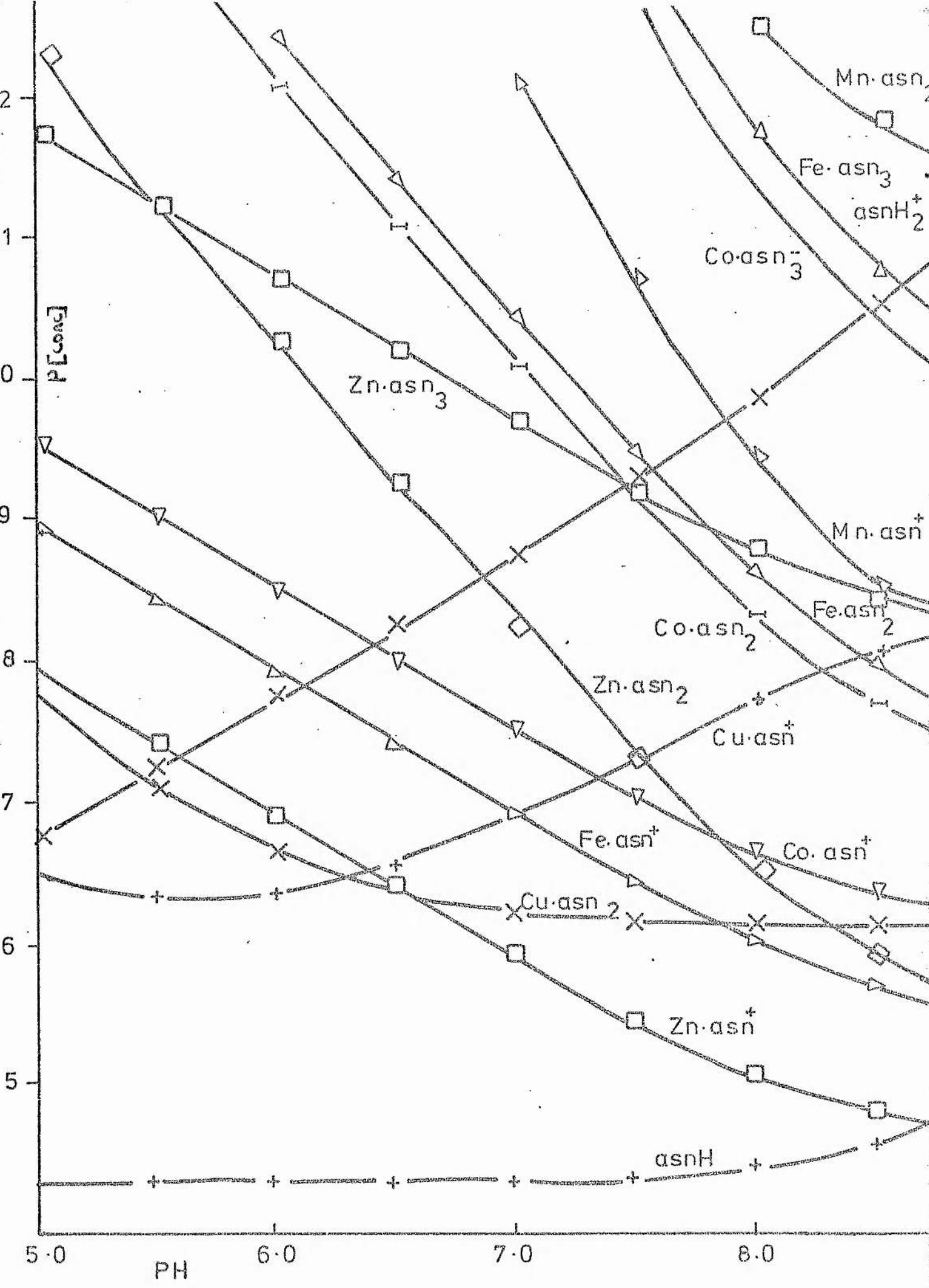


Figure 20 Simple model of Asparaginate in Blood Plasma

at relatively low concentrations in plasma.

Ternary complexes.

More and more workers have recently been dealing with the equilibria of mixed amino-acid complexes of the transition metals. The mixed complexes of Cu(II) are especially widely studied because of their biological importance. The equilibrium data of some ternary amino-acid complexes of Cu(II) were determined by Martin and Paris[145-146], who pointed out that the stabilities corresponded to the statistical expectations. On the other hand Freeman and Martin found significant stabilisation in the Cu(II)-histidinate-threoninate system[154]. More recently Sarkar and Kruck extended these studies to the Cu(II)-histidinate-serinate system[144]. The equilibrium data were interpreted as indicative of extra stabilisation in these systems. Yokoi et al[204] and Degisher and Nancollas[205] found that stabilities are determined mainly by entropy changes. Izatt et al[206] came to the conclusion that in some cases the stability is influenced by other factors.

Gergely et al[148] observed that amino-acid complexes of formula $CuAA^1$ displayed relatively higher stabilities when A was aliphatic and A^1 aromatic. This phenomenon was attributed to the electron-attraction of the aromatic systems, as a result of which the stability-increasing effect of the higher electron density on the nitrogen donor atoms of the aromatic amino-acids can exert itself.

Various methods of calculating the statistical factor in the stability

of ternary complexes have been proposed. Watters[207-208] pointed out that the formation of a ternary complex ABA^1 from a metal . .B in the presence of equal concentrations of ligands A and A^1, is always more favoured on a statistical basis than is A_2B or A^1_2B. These ternary complexes may be, and often are, more stable than expected from solely statistical effects[97].

Ligand-ligand interaction in a ternary species can also lead to destabilisation, i.e. the complex is less stable than expected after correcting for statistical effects[147]. In extreme cases the stability of ternary complexes are several times greater than that expected from purely statistical factors[209], presumably as a result of electrostatic, steric and hydrogen bonding phenomena. Bjerrum[167] divided ligand effects into a statistical, electrostatic and "reset" effect, the last-named constituting all contributions to the formation constants which cannot be explained either statistically or electrostatically.

Watters[208] calculated the statistical effect for mixed complexes involving two ligands as follows; With equal concentrations of ligands A and A^1 the probability that the first bonded ligand will be A is $\frac{1}{2}$. The probability that another A will bind next to form A_2B is $(\frac{1}{2})^2$ or one in four, and the same applies if A^1 binds first followed by another A^1. This means that the chances of forming ABA^1 are twice as great as forming either A_2B or A^1_2B alone. This calculation becomes more complex when three or more ligands are involved.

Bjerrum[167] has calculated the statistical effect for complexes

involving only one ligand, i.e. AB, A_2B etc., and this method could, in principle, be applied to ternary systems. A more general approach for calculating statistical factors can be expressed in the following manner : In aqueous solution containing a divalent metal ion B(II) and n different monodentate ligands A in equimolar concentrations, the highest mixed and simple complexes that can be formed are $BA_Q{}^q A_R{}^r . A_S{}^s . A_T{}^t . A_U{}^u . A_V{}^v$ and $BA_6{}^i$. The superscripts denote different ligands while subscripts Q, R, S, T, U, V, which are positive or zero, represent the number of corresponding ligands in the complex species.

The number of ways in which these species can be formed is given by,

$$N = \frac{n!}{Q!R!S!T!U!V!} \qquad (1)$$

Where n is the total number of ligands in the complex species. Thus the simple complex $BA_6{}^i$, containing six ligands of the same type can be formed in $6!/6!$ = one way only. On the other hand a mixed species containing six ligands all of different types can be formed in 720 ways. Equation (1) can be used to calculate the statistical factor for any mixed species.

From the following equation :

$$\log \beta_i = \frac{q}{Q} \log \beta_q (\text{for } BA_q) + \frac{r}{R} \log \beta_r (\text{for } BA_r^1) + \ldots + \log S \qquad (2)$$

the stability constant for the mixed complex can be calculated. q and r are the

numbers of ligands A, A^1 etc in the mixed complex, and Q and R are the numbers of ligands in corresponding simple complexes having the same metal-ligand ratio, while S is the statistical factor calculated from (1) (e.g. for a complex ABA^1 $\log S \simeq 0.301$).

Sharma and Schubert[210] have compared the calculated values of mixed complex formation with the experimental values along with the "ligand enhancement factor" ($\Delta \log \beta_i$) of the enhanced or diminished stability of the mixed complex, after correction for statistical effects. In mixed complexes of type ABA^1, $\Delta \log \beta_i$ is insignificant if A and A^1 are similar, However for mixed complexes of amines and amino-acids or carboxylic acids $\Delta \log \beta_i$ is always positive.

Another interesting phenomenon to be noted in the stability constants of ternary complexes is stereo-selectivity. Brookes and Pettit[156] measured constants for ternary complexes between copper(II), various amino-acids and either D- or L-histidinate. They found that in the cases of tryptophanate and phenylalaninate, the complexes with D-histidinate had formation constants significantly higher than the corresponding complexes with L-histidinate. No such effects were noted with amino-acids containing aliphatic side chains (valine, serine, threonine etc). For all the copper ternary complexes studied the stability constants were much higher than expected from purely statistical grounds. This appears to be a characteristic of the histidinate ligand.

Table 36 lists the thermodynamic parameters for the ternary species under investigation. Figures in parentheses are three times the standard deviation.

Table 36. Thermodynamic parameters for the ternary species

Complex	log β	$-\Delta G^{\circ}$ kJ mol^{-1}	$-\Delta H^{\circ}$ kJ mol^{-1}	ΔS° Jmol^{-1}K^{-1}	number of observations
Cu(II)thr^{+}	8.597 [0.007]	49.08 [0.04]	18.0 [1.5]	104.2 [5.0]	32
Cu(II)thr$_2$	16.031 [0.013]	91.53 [0.07]	47.0 [3.0]	149.4 [10.0]	
Cu(II).asn.thr.	16.471 [0.026]	94.04 [0.15]	50.0 [2.5]	147.7 [12.0]	16
Cu(II).asn.his.	18.597 [0.012]	106.15 [0.07]	67.5 [2.5]	129.7 [9.0]	20
Cu(II).asn.his.H^{+}	23.326 [0.031]	133.11 [0.17]	88.2 [5.0]	150.7 [17.5]	
Cu(II).his.thr.	18.613 [0.016]	106.22 [0.09]	67.4 [3.0]	130.3 [10.0]	20
Cu(II).his.thr.H^{+}.	23.426 [0.028]	133.68 [0.16]	103.5 [6.0]	101.3 [22.5]	

Freeman and Martin[154] have previously reported the complexes Cu(II).his.thr., Cu(II).his.H.thr.$^{+}$ and also Cu(II).his.thr.OH^{-}, observing that the constants for these species were larger than those of the parents, and that D as against L amino-acid stereospecificity was absent. The present work failed to detect the hydroxy complex, but values obtained for the other two species are in agreement with Freeman and Martin, after allowing for a change in the ionic background salt. Sarkar and Kruck[144] have studied the Cu(II).his.gln. system, which may be considered analogous to the present Cu(II).asn.his. study, and reported log β for the complexes Cu(II).gln.his (17.624) and Cu(II).gln.his.H^{+} (21.654), but no hydroxy species (c.f. Freeman and Martin). They also have studied the Cu(II)his.ser. system and found log β for the complexes Cu(II).ser.his (17.540) and Cu(II).ser.his.H^{+} (21.703). The Cu(II).asn.his. results in this work are comparable to the Cu(II).gln.his. results obtained by Sarkar and Kruck.

The two systems exhibiting protonated ternary complexes both contain histidinate, thus suggesting that the donor group protonated belongs to histidinate. Understandably, the chelating ability of all three ligands was pH dependent and the continuity of stepwise complexing was obeyed in that, for example Cu(II) ⟶ Cu(II)asn.his. via Cu(his)$^{+}$ and Cu(II)his.H^{2+}, all complexes being present in significant amounts in the pH range studied (see figure 17 Chapter 7).

The results exhibit the enhancement expected of ternary complexes, as seen in Table 37.

Table 37. Enhancement of the stability of ternary complexes compared to their parent binary complexes.

Complex	$-\Delta G^{o}$ (kJ mol^{-1}) Expt.	Calc.	difference	$-\Delta H^{o}$ (kJ mol^{-1}) Expt.	Calc.	difference	$T\Delta S^{o}$ JK^{-1} mol^{-1} Expt.	Calc.	difference
Cu(II)asn.his.	106.14	100.5 98.7	a	67.5	67.5 77.9	b	38.7	33.0 20.8	a
Cu(II)asn.his.H^{+}	133.09	130.	a	88.2	101.0	b	44.9	29.2	a
Cu(II)asn.thr.	94.04	92.0 90.2	a	50.0	56.5 52.0	b	44.0	35.5 38.2	a
Cu(II).his.thr.	106.22	100.0 100.1	a	67.4	72.9 58.0	c	38.8	27.1 42.1	c
Cu(II)his.H.thr^{+}	133.68	131.5	a	103.5	96.0	a	30.2	35.5	b

In table 37 a denotes expt> calc, b denotes expt< calc, and c denotes expt equal to, or between, calc values.

The approach used is to assume that there are two possible routes to forming, for example, Cu(II).asn.his. as in equations (1) and (2) below :

$$Cu^{2+} + asn. \xrightarrow{K_1^{asn.}} Cu.asn \xrightarrow{+\ K_2^{his.}} Cu.asn.his. \qquad (1)$$

$$Cu^{2+} + his. \xrightarrow{K_1^{his.}} Cu.his \xrightarrow{+\ K_2^{asn.}} Cu.asn.his. \qquad (2)$$

where the constants refer to the parent binary complex. Thus it is possible to obtain expressions (3) and (4).

$$\Delta G^{o}\left(Cu.asn.his.\right)(calc) = -RT\left\{\log \beta_1^{asn.} + (\log \beta_2^{his.} - \log \beta_1^{his.})\right\} \qquad (3)$$

$$\Delta G^{o}\left(Cu.asn.his.\right)(calc) = -RT\left\{\log \beta_1^{his} + (\log \beta_2^{asn.} - \log \beta_1^{asn.})\right\} \qquad (4)$$

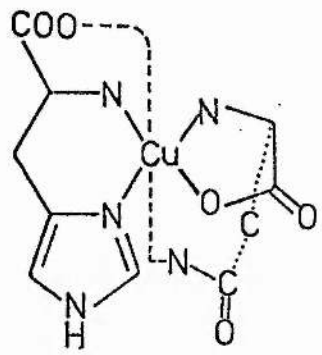
ΔH^{o}_{calc} and $T\Delta S^{o}_{calc}$ were computed likewise and are recorded in table 37. As seen from this table, an enhanced $-\Delta G^{o}$ arises from a lower than expected $-\Delta H^{o}$ and elevated $T\Delta S^{o}$ (see difference columns). The origins of these parallel and apparently related effects in ΔH^{o} and ΔS^{o} may now be traced to two possible effects. Ternary complex formation sometimes liberates more water from the solvation sphere of the copper ion (and sometimes less), thus increasing ΔS^{o} but requiring $-\Delta H^{o}$ to decrease because more aquation bonds are ruptured. Alternatively, ternary complex formation causes increased complex-bond strain. This, however, would result in lower $-\Delta H^{o}$ (a weaker complex bond) and a

lower $\triangle S^{o}$ (less degrees of freedom in the system). The former suggestion explains the $-\triangle H^{o}$, $\triangle S^{o}$ (b,a) pattern in Table 37, whereas the bond-strain suggestion would involve a b, b, or a, a pattern. However there is a need for more experimental data to verify this postulate.

Structure of the ternary species.

The most important details arising from this study are thermodynamic conclusions which suggest the structures of the complexes present in aqueous solution. Spectral investigations of structure perceive only those aspects of the structure that are involved in electronic transitions. On the other hand enthalpies and entropies of complex formation reflect all bond strengths, ring strains and configurations. In order to recognise these characteristics in $\triangle H^{o}$ and $\triangle S^{o}$ patterns the question of ternary structures ought to be approached via those of their parent binary complexes. The denticity of the amino acids has already been discussed (p.170) ; threoninate is bidentate, histidinate tridentate and asparaginate is possibly also tridentate. Each of the five ternary complexes will now be discussed in turn.

Cu(II).asn.his. The histidinate is shown bonded, as argued by Williams[27], with two planar amine-Cu(II) bonds and a longer axial carboxylate-Cu(II) bond. The asparaginate is shown as a transient tridentate ligand having the basic planar bonding to its amino-acid groups.

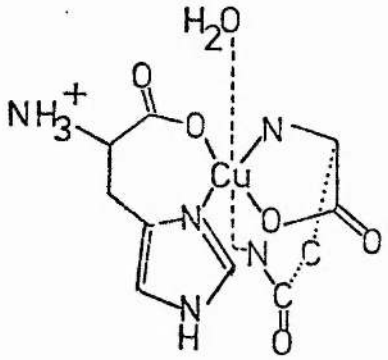


The question as to whether the NH_2 groups are cis or trans to each other is still open. However cis should be favoured, on the grounds of the repulsion of two negatively CO_2^- groups, the his. being axial and the asn. being planar. Were the bond configurations to be truly symmetrical Jahn-Teller octahedral, the distances between the carboxylate groups for both cis and trans suggestions would be similar. However molecular models suggest that the histidinate CO_2^- - Cu(II) bond is not at 90^o to the plane, but nearer to 70^o. Thus less intercarboxylate repulsion occurs for the cis-NH_2 model. Another question is whether the asparaginate side-group bonded in extended axial position is through the NH_2 or the C = O. As illustrated above, a Cu-N bond is tentatively suggested, because Cu(II) prefers N rather than O donors. The overall test that the basic structure is as drawn is that calculated values of ΔH_1^o and ΔS_1^o, calculated on the assumption that binary structures survive in the ternary complexes, are in agreement with the experimentally observed values in Table 37.

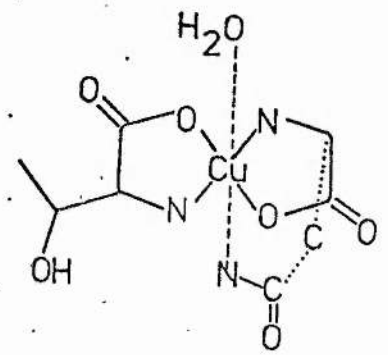
Cu(II).asn.his.H^+ Only ternary complexes which contain histidinate form protonated species. This shows that the site of protonation is at one

of the three donor groups of histidinate. Furthermore, the binary Cu(II).asn. system does not have protonated species.

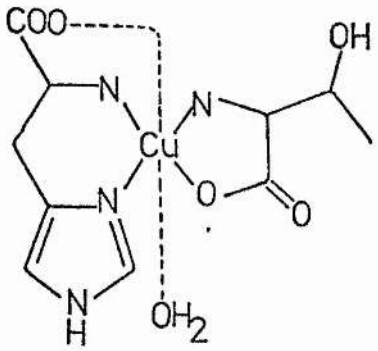
The proton is assumed to be attached to the primary amine of histidinate[27], the axial CO_2^- group moving inwards towards the central metal ion to adopt a planar bonding position <u>trans</u> to the asparaginate CO_2^- group. The enthalpy for the reaction Cu(II).asn.his + H^+ ⟶ Cu(II).asn. his.H^+ (20.7 kJ mol^{-1}) is comparable to that of Cu(II).his$^+$ + H^+ ⟶ Cu(II).his.H.$^{2+}$ (23.1 kJ mol^{-1}).



<u>Cu(II).asn.thr.</u> The Cu(II)-asparaginate bonding is as just described, and the threoninate is bidentate as deduced before. The hydroxyl group on threoninate hangs free, and is not bonded to the Cu(II). The principle of repulsion between like groupings suggests that the carboxylate and primary amine groups are <u>trans</u> to each other.

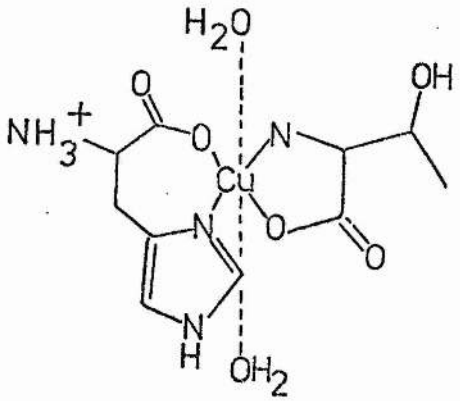


Cu(II).his.thr. The suggested aqueous structure of this complex is based on the binary parent logistics previously advanced for histidinate and threoninate.



Sarkar et al[211] suggested that histidinate bonded like threoninate, via carboxylate and primary amine group. However, the crystal structure has been determined by Freeman et al[212], and their findings are in agreement with the proposed structure. The two $-NH_2$ groups are cis and the angle $N(NH_2)$ - Cu - O (CO_2^-) is 68.3^o in the crystal structure.

Cu(II).his.H.thr$^+$ The suggested aqueous structure of this cationic complex is shown below. The proton is attached to the primary amine of histidinate, and the other features have been deduced as for the previous ternary complexes.



This section of the present work has examined the relatively new field of ternary-structure determination in aqueous solution and has demonstrated that precision thermodynamic measurements can provide many of the important bonding details. "Precision" encompasses the best methods available for ΔG° determination (both data acquisition and least squares computation) and then accurate calorimetry involving reliable ΔH° corrections (for the other complexes formed simultaneously to the ternary ones) to obtain ΔH° and ΔS°. The "structures" are then revealed through bond strengths, ring strains and some configurational information. This ought to be contrasted with X-ray crystallographic determinations which reveal bond angles, dimensions and configurations for the non-aqueous crystalline state. The two methods are complementary, giving different views of the same system under different ambient conditions.

Conclusions and future work.

The body is only 3% metals by weight, yet the significance of metals in life is far greater than this low figure would indicate. For example, metal ions are often involved when amino-acid units are being built up into proteins.

The present work has dealt with the complexation of asparaginate, a simple ligand, yet it forms a large number of metal complexes. Future work should include the study of the complexation of asparaginate with Fe(III) where hydroxy species are almost certainly involved, and a method for obtaining calorimetric data for these as well as for the Fe(II) complexes

needs to be developed.

The latter part of this work discussed a number of ternary systems involving Cu(II) and two different amino-acids. These systems have been shown to be important in copper transport, and future work in this field should be extended to include ternary species with other metal ions. Fe(III) seems to be a particularly good candidate, for ternary complexes with this metal ion may well be involved in iron transport. The *in vitro* study of systems of this type will help us to understand the *in vivo* chemistries of the same systems.

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